Docket No.: 2585-0126PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Claudia Angelica SOTO PEREDO

Application No.: 10/538,277 | Confirmation No.: 9420

Filed: June 10, 2005 | Art Unit: 1614

For: PHARMACEUTICAL COMPOUND CONTAINING SILYMARIN AND CARBOPOL, PRODUCTION METHOD THEREOF AND USE OF SAME AS A REGENERATOR OF TISSUE AND PANCREATIC CELLS WITH ENDOGENOUS SECRETION DAMAGED BY DIABETES MELLITUS | Examiner: Z. Vakili

DECLARATION UNDER 37 C.F.R. § 1.132

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Claudia Angelica SOTO PEREDO, Ph.D., do declare and say as follows:

1. I am the inventor of above-identified application.

2. I am currently a full researcher-professor in the Course of Pharmacology of Postgraduate Course Interaction Drug-Organism and Experimental Drug Evaluation in the Biological Systems Department at the Universidad Autónoma Metropolitana. A copy of my *curriculum vitae* is attached.

3. I have read the Final Office Action dated May 30, 2008 in the above-identified application and understand its contents.

4. I have read and understand the contents of the references cited in the May 30, 2008, Office Action, which are the references of:

- **Bibbs *et al.* '128** (U.S. Publication No. 2004/0006128 A1);
- **Soto *et al.* (1998)** (*Comp. Biochem. Physiol.*, Vol. 119C, No. 2, pp. 125-129 (1998)); and
- **Coote *et al.* '034** (U.S. Publication No. 2004/0167034 A1).

5. In the May 30, 2008, Office Action, the Examiner refers Applicant to various parts of Bibbs *et al.* '128, Soto *et al.* (1998) and Coote *et al.* '034. However, one of skill in the art would understand that lowering blood glucose does not lead to the regeneration of the endocrine pancreatic function, and that the presently claimed invention is directed to a use that is not explicitly or inherently disclosed in the cited references.

6. Alloxan causes necrosis of pancreatic β-cells, thereby leading to a lack of insulin secretion, and induces oxidant free radicals.

I have conducted experimentation to show how the claimed invention works using alloxan to induce experimental diabetes mellitus in rats. This experimentation is reported in the attached article wherein I am a co-author: C. Soto *et al.*, "Silymarin induces recovery of pancreatic function after alloxan damage in rats," *Life Sciences*, Vol. 75, pp. 2167-2180 (2004)

(hereinafter "*Life Sciences* (2004)"). The aim of the study was to test whether silymarin could reduce hyperglycemia and revert pancreatic damage in alloxan treated rats. There were two protocols for testing: administration of alloxan and silymarin simultaneously for 4 or 8 doses; or administration of silymarin 20 days after alloxan administration for 9 weeks.

Animal treatments of alloxan and silymarin

Two protocols: use of alloxan and silymarin simultaneously for 4 or 8 doses; and use of silymarin 20 days after alloxan administration for 9 weeks. Serum glucose and insulin were determined, and pancreatic fragments were used for histology and insulin immunohistochemistry.

As stated on page 2169 and summarized in Table 1 (page 2171), male Wistar rats were divided in Groups 1)-9) as follows:

Group 1): control group with no drug treatment;

Group 2): administration of silymarin only, wherein silymarin is administered at 0, 6, 24 and 48 hours;

Group 3): administration of alloxan only (single dosage);

Group 4): administration of silymarin plus alloxan, wherein silymarin is administered at 0, 6, 24 and 48 hours (like Group 2) and a single dosage of alloxan (like Group 3);

Group 5): administration of silymarin plus alloxan like Group 4) except Silymarin administered every 24 hours for 7 days and single dosage of alloxan;

Group 6): administration of silymarin plus alloxan, except silymarin administered every 24 hours for 9 weeks and single dosage of alloxan.

Group 7): the control group of Group 6) wherein only vehicles of silymarin and alloxan are administered;

Group 8): like Group 6) except no administration of alloxan;

Group 9): administration of a single dosage of alloxan and after 20 days administration of carbopol for 9 weeks.

Obtained results: glucose levels

Previously, in our article Soto *et al.* (1998), my colleagues and I had demonstrated that silymarin prevented alloxan-induced diabetes mellitus in rats and such an effect was due to the antioxidant properties.

As reported in the attached *Life Sciences* (2004) study, after 72 hours after alloxan administration, serum glucose increased and serum insulin decreased significantly. Silymarin produced a recovery of insulin serum levels in alloxan pancreatic damage in rats. For instance, notable increases in serum glucose levels to match levels seen in the control group were observed after seven days of silymarin treatment (see page 2171 under "Results"). However, administration of silymarin only did not change serum glucose (page 2172, lines 5-6).

Obtained results: recovering endocrine pancreatic function

Normal rat pancreas tissue morphology is depicted in Figure 3A (see discussion starting at the bottom of page 2172). Figure 3B shows damaged pancreatic tissue after alloxan administration.

However, upon simultaneous treatment of silymarin with alloxan for 48 hours and as seen in Figure 4A, no pancreatic tissue morphological difference was observed compared to the normal, non-treated rat control groups (see page 2173, first full paragraph). Similar observations were made with treatment of alloxan for 5 days (Figure 4B). Even after 30 days, the pancreatic tissue of the rats of Group 5) was unaffected (Figure 4C). Figure 4D shows silymarin administration for 9 weeks starting after 20 days of alloxan administration, wherein the treated tissue was undistinguishable from normal, non-treated rat controls.

Thus, silymarin recovered the endocrine pancreatic tissue in alloxan-induced diabetes mellitus affected rats at both structural and functional levels (see the "Discussion" section at pages 2176-2177). The weight, size and physical characteristics of rats during recovery after silymarin treatment were similar to normal control animals (see page 2177 of the attached article). The pancreas of rats treated with silymarin presented evidence of β-cell recovery from the third day when silymarin was administered on the first day of alloxan treatment (see page 2178 of attached article). Silymarin also increased the survival rate just after three days of administration (see Table 2 on page 2178 and page 2175 under "Animal survival").

Claimed invention versus Bibbs *et al.* '128, Soto *et al.* (1998) and Coote *et al.* '034

The claimed invention is directed to a method of recovering endocrine pancreatic function in a patent in need thereof, wherein the method comprises orally administering to the patient an effective amount of a composition comprising a mixture of silymarin and carbopol, thereby recovering endocrine pancreatic function by regenerating damaged pancreatic cells after the administration.

As can be seen from the *Life Sciences* (2004) article, lowering blood glucose does not mean regeneration of the endocrine pancreatic function. The Soto *et al.* (1998) article demonstrates silymarin's antioxidant properties on alloxan-induced diabetes mellitus rats. The *Life Sciences* (2004) study demonstrates that silymarin recovers endocrine pancreatic tissue in alloxan-induced diabetes mellitus affected rats at both structural and functional levels, including survival rates. The pancreatic histological examination as depicted Figures 4A-4C shows how silymarin treatment can lead to regeneration of the tissue such that it becomes indistinguishable from the tissue of the normal, non-treated control groups.

Bibbs *et al.* '128 only mentions that blood glucose is lowered but does not cite that β-pancreatic cells are regenerated. As explained above, the lowering of blood glucose in Bibbs *et al.* '128 does not necessarily entail that β-pancreatic cells are regenerated.

In the May 30, 2008 Office Action, the Examiner asserts that Soto *et al.* (1998) teaches that silymarin has shown protective effects against the oxidative peroxidation of cells. Soto *et al.* (1998) shows silymarin's non-regenerative protective effect. As explained above, the lowering of blood glucose does not necessarily entail that β-pancreatic cells are regenerated.

As further support that the lowering of blood glucose does not necessarily entail that β-pancreatic cells are regenerated, *Chapter 60* of "Insulin, Oral Hypoglycemic Agents, and the Pharmacology of the Endocrine Pancreas," of *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th Edition is herein attached. *Chapter 60* of *The Pharmacological Basis of Therapeutics* extensively discusses and differentiates between lowering blood glucose versus other functions, including regeneration of the endocrine pancreatic function.

Disclosure of a bioflavonoid does not equate to disclosure of regeneration of pancreatic function

Further, the disclosure of a bioflanovoid in a publication having the property of lowering blood glucose does not mean that particular bioflavonoid will inherently regenerate pancreatic function. Attached is a review article by A. Andrade-Cetto *et al.*, "Mexican plants with hypoglycaemic effect used in the treatment of diabetes," *Journal of Ethno-Pharmacology*, Vol. 99, pp. 325-348 (2005).[1] The Andrade-Cetto *et al.* article discusses how a multitude of bioflavonoids found in Mexican plants (see Table 1 that spans pp. 328-336) can have different pharmacological effects and that it is not predictable as to whether or not all flavonoids can have the ability to lower the blood glucose, regenerate endocrine pancreatic function and/or other properties.

Thus, one of skill in the art would understand that lowering blood glucose does not lead to the regeneration of the endocrine pancreatic function, and that the presently claimed invention is directed to a use that is not explicitly or inherently disclosed in the cited references of Bibbs *et al.* '128, Soto *et al.* (1998) and Coote *et al.* '034.

[1] Though this scientific article is dated 2005, this article is a review article and p. 346 lists a multitude of scientific references that pre-date the priority date of the present patent application.

7. I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11/21/2008 By: _____
Claudia Angelica SOTO PEREDO

Attachments:

- *Curriculum vitae*
- C. Soto *et al.*, "Silymarin induces recovery of pancreatic function after alloxan damage in rats," *Life Sciences*, Vol. 75, pp. 2167-2180 (2004)
- Andrade-Cetto *et al.*, "Mexican plants with hypoglycaemic effect used in the treatment of diabetes," *Journal of Ethno-Pharmacology*, Vol. 99, pp. 325-348 (2005)
- *Chapter 60* of "Insulin, Oral Hypoglycemic Agents, and the Pharmacology of the Endocrine Pancreas," of *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (11th Ed.)

CURRICULUM VITAE

NAME: **CLAUDIA ANGELICA SOTO PEREDO.**

EDUCATION:

University Degree: Pharmaceutical Industrial Chemist (National School of Biological Sciences, 1979).

Postgraduate course:

Master of Science in Pharmacology (Center of Research and Advanced Studies of the National Polytechnic Institute (I.P.N.), 1982).

PhD. of Science in Pharmacology (Center of Research and Advanced Studies of the National Polytechnic Institute (I.P.N.), 1995).

WORK EXPERIENCE IN INDUSTRY:

Analyst in Medical Laboratories of Chopo (1973).

Chemical Analyst in Quality Control and Development of New Formulas
Pharmaceutical Laboratories Galén (1975-1976).

Chemical Analyst in Quality Control - Laboratories "Pharmaceutical Corporation" (1976).

WORK EXPERIENCE IN TEACHING:

Professor of Subject "A" of Pharmacology in E.N.E.P., "Zaragoza" of the U.N.A.M. (1978-1979).

Participation as a Professor in the Course of Renal Physiology and Pharmacology of Postgraduate Course in the Physiology, Biophysics and Neuroscience Department of Center of Research and Advanced Studies of the I.P.N. (199-1997).

UAM-Xochimilco:

Associate Professor "D" Full-Time (1980 to July, 1992).

Promotion to Full Professor "A" (July 27, 1992).

Promotion to Full Professor "B" (October, 1993 to December, 1995).

Promotion to Full Professor "C" (January, 1996 to present).

Plan of Course: "Specialization in Pharmacology" (1980-1981)

Teaching of Module: "Experimental Evaluation of Drugs" in the career of Chemical-Pharmaceutic Biologist (1983 to present).

Teaching of Module: "Drug-Organism Interactions" in Master of Pharmaceutical Sciences (1999 to present).

Teaching of Workshops: Chemistry I and Maths I (1985 to 1987).

Coordination of Course: "Pharmacodynamics" (1983).

Reorganization of Workshops of Physical-Chemistry and Statistical Study of Experimental Evaluation Module of Drugs.

Reorganization of Module: Experimental Evaluation of Drugs.

Plan of Master in Pharmaceutical Sciences (1999).

Reorganization of the Chemical-Pharmaceutic Biolgist career. (1999-2000).

Development of research project "Mechanism of Blood Transport and Action Mode of Alloxan" (1989 to 2004).

Development of research project "Effect of Silymarin on the Diabetes Mellitus and its complications" (1993 to present).

SUPERVISED THESIS OF BACHELOR'S DEGREE

Effect of alloxan on the activity of dependent sodium-potassium pump in skin of *Rana Pipiens*. Areli Ramírez Moreno and Fernando Paz Osuna. March, 1991.

Temporal course of plasmatic levels of glucose and glutathione and pancreatic lipoperoxidation in experimental diabetes mellitus. Rocío Pérez Sánchez and Jesús Calderón Padilla. December, 1991.

Effect of 3', 4'-dicholorobenzamil in relation to Na+/Ca2+ in an experimental model of diabetes mellitus. María Guadalupe Aguilar Aguila. June, 1995.

Effect of quinacrine in relation to Na+/Ca2+ in an experimental model of diabetes mellitus. Lorena Guadalupe Jiménez Clavel. June, 1995.

Effect of Silymarin in the establishment of diabetes mellitus induced by alloxan in rat. Blanca Pérez López. September, 1995.

Effect of vitamin E on the temporal course of establishment of diabetes mellitus by alloxan in rat. Luz María Hernández Ramírez. January, 1996.

Intracellular content of K^+ in the isolated proximal tubule of rat with diabetes mellitus. José Luis Lozada González. October, 1998.

Intracellular content of Na^+ in the isolated proximal tubule of rat with diabetes mellitus. Lorena Zúñiga Flores. October, 1998.

Effect of Silymarin in the renal content of glutathione during the induction of experimental diabetes mellitus of rat. Sagrario Tovar Franco. December, 1998.

Effect of tetraethylammonium on the decrease in sodium transport produced by alloxan in epithelium of frog skin. Marbin Zúñiga Rodríguez. December, 1999.

Effect of Silymarin in renal lipoperoxidation degree during the induction of experimental diabetes mellitus in rat. José Antonio Barrera Elizarrarás. May, 2000.

Effect of Silymarin on induced diabetes mellitus in rat pancreas. Maribel Sánchez Ramos. September, 2000.

Effect of Silymarin in the renal peroxidase glutathione activity during the induction of experimental diabetes mellitus in rat. José Antonio Velázquez Aragón. January, 2001.

Effect of Silymarin in the renal dismutase superoxide activity during the induction of experimental diabetes mellitus in rat. Iván García Villegas. September, 2001.

Histopathologic Study of the effect of Silymarin on a diabetic nephropathy model. Pedro Antonio de la Rosa. November, 2001.

Effect of Silymarin on the pancreatic peroxidase glutathione activity in experimental diabetes mellitus in rat. Héctor Barrón Cuevas. May, 2003.

Effect of Silymarin on the pancreatic catalase activity in experimental diabetes mellitus in rat. Rosa María Recoba Lara. July, 2003.

Effect of Silymarin on the serumal level of insulin in diabetes mellitus model. Marcela Victoria Cortés. September, 2003.

Effect of Silymarin in the pancreatic dismutase superoxide activity in the experimental diabetes mellitus in rat. Carlos Alvarez. January, 2004.

Modification of vascular reactivity induced by Diabetes Mellitus. Omar Echeverría Rodríguez. April, 2007.

Annual report of spontaneous reporting received by the National Pharmacovigilance Centre. César Alik Pedrajo Zenteno. December, 2007.

Effect of Silymarin on the proliferation of β-pancreatic cells in rats partially pancreatectomized. Edna Jazmín Trejo Escalante. June, 2008.

CONSULTANCY OF POSTGRADUATE THESIS

Participation of oxidative damage in endocrine pancreas function of rats subchronically treated with arsenite. Jeannett Alejandra Izquierdo Vega. Master in Toxicology CINVESTAV, IPN. March, 2004.

Effect of the polysaccharide obtained of Tamarindus indica linn. pulp in corporal temperature and its relation with the production in Vitro of IL-1β, IL-6 and PGE_2 in mice Balb/C. Phd. in Biological Sciences UAM. October, 2007.

CO-TUTORSHIP OF POSTGRADUATE THESIS

Analysis of initial stages of amyloidogenic process of tau protein in neurofibrillary degeneration in Alzheimer's disease. Study by confocal microscopy. José Carmen Luna Muñoz. PhD. in Biological Sciences UAM. February, 2005.

PROJECTS EXHIBITED IN CONGRESS

1st. Student Scientific Conferences of National School of Biological Sciences (Mexico, Federal District, 1975). Acute effect on learning of anti-epileptic 5-ethyl.5.phenyl-2-pyrrolidone (EPP). Soto Peredo Claudia and Chambert Castillo Guillermo.

III National Congress of Pharmacology (Morelia, Mich., 1979). Determination of medium lethal dose of trimethylentrinitramine in rats. Pedro A. Lehman F., E. Cardona, L. Favari, L. Gómez, B. Mendiola, M.C. Ramírez, C. Soto and L. Trujillo.

II Meeting of the CESTC (Center of Studies on Connective Tissue). (La Trinidad, Tlaxcala, 1986). Covalent bonds of normal and cirrhotic liver. Claudia Soto and Patricia Greenwel.

37th Annual Meeting of the American Association for the Study of Liver Diseases (Chicago, III., USA, 1986). The reducible cross links of liver collagen: Changes with aging and cirrhosis. C. Soto and M. Rojkind.

II Congress of Research of Biological Systems Department of the Metropolitan Autonomous University (Oaxtepec, Mor., 1989). Effect of alloxan on the activity of dependent sodium-potassium pump in skin of Rana Pipiens. Claudia Soto, Cuauhtémoc Pérez, José Luis Reyes, Areli Ramírez and Fernando Paz.

XIII National Congress of Pharmacology (Pátzcuaro, Mich., 1989). Effect of demecolcine on induction of experimental hepatic cirrhosis. Soto, C., Mourelle, M. and Pérez, V.

XXXII National Congress of Physiological Sciences (Oaxtepec, Mor., 1989). Effect of alloxan on sodium transport in skin of Rana Pipiens. Soto, C., Reyes, J.L., Pérez, C., Ramírez, A. and Paz, F.

III Congress of Research of Biological Systems Department of the Metropolitan Autonomous University (Oaxtepec, Mor., 1990). Temporal course of alloxan effects on levels on glucose, glutathione and lipoperoxidation degree in mice CD-1. Soto, C., Pérez, R., Reyes, J.L., Mourelle, M.

XIth International Congress of Pharmacology (Amsterdam, The Netherlands, 1990). Alloxan decreases transepithelial potential difference and short circuit current in the isolated frog skin. Soto, C., Reyes, J.L., Ramírez, A., Paz, F. and Pérez C.

XXXIII National Congress of Physiological Sciences (Guadalajara, Jal., 1990). Temporal course of alloxan effects in mice CD-1. Soto, C., Pérez, R., Reyes, J.L., Mourelle, M.

XXXIV National Congress of Physiological Sciences (Colima, Col., 1991). Participation of calcium in the inhibitory effect of alloxan on sodium transport in skin of Rana Pipiens. Soto, C., Reyes, J.L., Pérez, R. and Pérez, C.

XXIV National Congress of Physiological Sciences (Marina, Vallarta, Jal., 1991). Antimicrobial action of the Sellaginella Lepidophylla. Claudia Soto Peredo, Angélica Salomón Serna, Salud Pérez Gutiérrez.

XXXV National Congress of Physiological Sciences (Veracruz, Ver., 1992). Analysis of interaction of calcium and sodium on the alloxan effect in the skin of Rana Pipiens. Soto, C., Reyes, J.L., Arteaga, R. Jiménez, L., Rodríguez, C.

IV Congress of Research of Biological Systems Department (Oaxtepec, Mor., 1993). Mechanism of transport in blood and mechanism of action of the alloxan. Soto, P.C.A., Reyes S.J:L.

International Symposium on The Lipid Triad (Triglycerides, HDL, LDL) and Cardiovascular Diseases (Milan, Italy, 1993). Glutathione and pancreatic lipid peroxidation in alloxan-induced diabetes mellitus. Claudia Soto, Pablo Muriel, José L. Reyes.

II Latin American Congress of Biophysics (Puebla, Pue., 1993). Inhibition of sodium transport by alloxan. C. Soto, J.L. Reyes, L. Jiménez and G. Aguilar.

XII International Congress of Pharmacology (Montreal, Canada, 1994). Effect of quinacrine and dichlorobenzamil on the sodium transport in the frog skin. Soto C., Jiménez L., Aguilar G. and Reyes J. L.

6th International Symposium on Pharmacological Control of Calcium and Potassium Homeostasis (Florencia, Italy, 1994). Role of a Na+/Ca2+ exchanger, calcium entry and chloride in the transepithelial sodium transport. Soto C., Reyes J.L.

XXXVII National Congress of Physiological Sciences (Mérida, Yuc., 1994). Participation of sodium/calcium exchanger in modulation of sodium transport and in chloride secretion in isolated skin of frog. Soto Claudia, Jiménez Lorena, Sierra Gerardo and Reyes José Luis.

XXXVIII National Congress of Physiological Sciences (Querétaro, Qro., 1995). Effect of Silymarin on changes produced by alloxan in induction of diabetes mellitus in rat. Pérez B. and Soto C.

XXXVIII National Congress of Physiological Sciences (Querétaro, Qro., 1995). Dependence of Na+-Ca2+ exchanger in the increase of sodium induced by oxytocin. C. Soto, G. Aguilar, L. Jiménez and J.L. Reyes.

XXXIX National Congress of Physiological Sciences(Puebla, Pue., 1996). Effect of alloxan on the ATPase activity dependent of Na+ and K+ in epithelium isolated of *Rana Pipiens* skin. Soto C., Sierra G., Reyes J.L., Escalante B. and Del Razo L.M.

V Research Congress of Biological Systems Department UAM-X (Taxco, Gro. 1996). Mechanism of Blood Transport and Action Mode of Alloxan. Soto P.C.A., Reyes S.J.L.

XLV Annual Meeting of the Mexican Institute of Nephrological Research, A.C. (por sus siglas en español, *Instituto Mexicano de Investigaciones Nefrológicas, A.C.)* (Huatulco, Gro. 1996). Effect of an inductor of diabetes mellitus (alloxan) on the content of intracellular potassium in isolated epithelium. Claudia Soto and José L. Reyes.

XL National Congress of Physiological Sciences (Morelia, Mich., 1997). Effect of glibenclamide and of tetraethylammonium on the change induced by the alloxan in the intracellular contenido of potassium in the isolated epithelium of the frog skin. Soto C., Sierra G., Del Razo L.M. and Reyes J.L.

XL National Congress of Physiological Sciences (Morelia, Mich., 1997). Study by confocal microscopy of the fluorescence in collector tubules of a newborn and adult rabbit loaded with Fluo3. Namorado M.C., Martín D., Sierra G., Soto C. and Reyes J.L.

XL National Congress of Physiological Sciences (Morelia, Mich., 1997). Effect of glibenclamide and of tetraethylammonium on the decrease in sodium transport produced by alloxan in epithelium of frog skin. Rojas M., Zúñiga M., Urbina M. and Soto C.

XLV Annual Meeting of the Mexican Institute of Nephrological Research, A.C. (Acapulco, Gro. 1998). Effect of Silymarin on lipoperoxidation and renal concentration of glutathione in diabetes mellitus induced by the alloxan in rat. Claudia Soto, Sagrario Tovar and José Antonio Barrera.

ACS Pan-American Conference. (Puerto Rico, 2000). Study of a drug with therapeutic potential on diabetes mellitus. Claudia Soto and José Antonio Velázquez.

XI Scientific Research Seminar in UAM-Xochimilco. (Mexico, Federal District, 2001). Effect of Silymarin in the activity of two pancreatic antioxidant enzymes in experimental diabetes mellitus in rat. Carlos Alvarez Contreras, Héctor Barrón Cuevas, Marcos Mendoza Mejía, Rosa María Recoba Lara and Claudia Soto Peredo.

XLIV National Congress of Physiological Sciences (Monterrey, Mexico, 2001). Effect of Silymarin on peroxidase glutathione and dismutase superoxide in experimental diabetes mellitus in rat. Soto Peredo C. Barrón Cuevas H., Mendoza Mejia M., Recoba Lara Rosa M.

XXXIV National Congress of Pharmaceutical Sciences (Manzanillo, Mexico, 2001). Antioxidant effect of Silymarin on pancreas in experimental diabetes mellitus in rat. Soto P. Claudia, Barrón C. Héctor, Mendoza M. Marcos, Recoba L. Rosa Ma., Alvarez C. Carlos.

VII Departmental Congress of Biological Systems (Oaxtepec, Mexico, 2001). Effect of Silymarin on antioxidant system in experimental diabetes mellitus in rat. Claudia Soto and Marcos Mendoza.

XIVth World Congress of Pharmacology. (San Francisco, California, USA, 2002). Silymarin effect in pancreatic activity of antioxidant enzymes in experimental diabetes mellitus of the rat. C. S. Peredo, R. Recoba, H. Barrón, C. Alvarez.

XLV National Congress of Physiological Sciences (Colima, Mexico, 2002). Effect of Silymarin on renal activity of peroxidase glutathione and dismutase superoxide in the induction of experimental diabetes mellitus in rat. Relation with a histopathological study. Soto Peredo, C. Uría Galicia, E., De la Rosa Oropeza, P., García Villeda, I., Velázquez Aragón, J.

LI Annual Meeting of the Mexican Institute of Nephrological Research. (Acapulco, Mexico, 2002). Effect of Silymarin on the renal activity of two antioxidant enzymes in diabetes mellitus induced by alloxan in rat. Claudia Soto, Esther Uría, José A. Velázquez A., Rosa M. Recoba, Héctor Barrón, Carlos Alvarez.

XII Scientific Research Seminar in UAM-Xochimilco. (Federal District, Mexico, 2003). Protective effect of Silymarin on renal damage in diabetes mellitus. Claudia Soto, Héctor Barrón, Rosa Ma. Recoba.

18th International Diabetes Federation Congress. (París, Francia, 2003). Silymarin regenerates pancreatic tissue damaged by Alloxan in hyperglycemic rats. C. Soto, R. Mena, J. Luna, E. Uría, R. Recoba, H. Barrón, M. Victoria, E. Larrieta, P. Vital, A. Lara.

CINVESTAMBIENTE 2003. (Federal District, Mexico, 2003). Oxidative pancreatic damage produced by chronic exposure to arsenite in rats. Jeannett Izquierdo-Vega, Claudia A. Soto and Luz M. del Razo.

43rd Annual Meeting Tox. Expo. Society of Toxicology. (Baltimore, USA. March, 2004). Pancreatic oxidative damage in endocrine function in rats subchronically exposure to arsenite. Jeannett Izquierdo-Vega, Claudia A. Soto and Luz M. del Razo.

XXV Congress of Mexican Community of Biochemistry. (Ixtapa, Gro. Dec. 2004). Effect of Silymarin on the pancreatic endocrine function in a model of diabetes mellitus. Soto Peredo C., Mena López R. Luna Muñoz J., Cerbón Cervantes M.A.

XXV Congress of Mexican Community of Biochemistry. (Ixtapa, Gro. Dec. 2004). Resistance to insulin in rats subchronically exposed to Arsenite. Izquierdo-Vega, J.A. Soto-Peredo, C., Sanchez-Peña, L.C. and Del Razo, L.M.

2nd Meeting of Students and Graduates of the Master of Pharmaceutical Sciences (Mexico, Federal District, Dec., 2005). Effect of silymarin on the expression of transcription factors Pdx1, Foxa-2 and Nkx6.1 in the development of β-pancreatic cells. Alvarez Contreras Carlos, Gómez Rico Jacobo, Montiel Hernández Claudia, Pérez Ramos Julia, Sainz Espuñes Teresita, Noguez Méndez Norma, Soto Peredo Claudia.

2nd Meeting of Students and Graduates of the Master of Pharmaceutical Sciences (Mexico, Federal District, Dec., 2005). Dissolution profile of sodium Naproxeno, pills of 550 mg. of two trademarks. Recillas Morales Sergio, Noguez Méndez Norma, Soto Peredo Claudia.

2nd Meeting of Students and Graduates of the Master of Pharmaceutical Sciences (Mexico, Federal District, Dec., 2005). Studies of Metronidazol by dissolution profiles. Nava Sánchez Artemio, Ramírez Lubianos Concepción, Soto Peredo Claudia, Noguez Méndez Norma.

2nd Meeting of Students and Graduates of the Master of Pharmaceutical Sciences (Mexico, Federal District, Dec., 2005). Comparative study of dissolution profiles of pills of 400 mg of ibuprofen of three trademarks. Cruz Ruiz Gabriela, Hernández Oreano Eric, Noguez Méndez Norma, Soto Peredo Claudia.

Meeting of Toxicology. Toxicology and Health in Mexico. Breakthrough and Perspectives (Mexico, Federal District, Nov., 2003). Deficiency role of selenium in hypoglycemia and

hyperinsulinemia caused by chronic exposure to arsenite in rats. Izquierdo-Vega, J.A. Soto, C.A., Sánchez-Peña, L.C. and Del Razo, L.M.

V National Congress AMCAL and III Central American Meeting and of Caribbean AMCAL-ACCMAL-ICLAS (ARC). (Huatulco, Oaxaca, Dec. 2005). Distribution of arsenical species in rat pancreas subchronically exposed to arsenite. Izquierdo-Vega, J.A., Sánchez-Peña, L.C., Barrera-Hernández, A. and Del Razo, L.M.

XVth International Congress of Pharmacology. (Beijing, China, Junio, 2006). Effect of Silymarin in the proliferation of pancreatic β-cells in experimental diabetes mellitus at early stages of the treatment. Soto Claudia, Trejo Edna, Fernández Juana, Uría Esther, Luna José, Mena Raúl, Pérez Julia.

XVth International Congress of Pharmacology. (Beijing, China, Junio, 2006). Effect of Silymarin in the pancrreatic transcription factors RNAm expression in experimental diabetes mellitus at early stages of the treatment. Pérez Julia, Alvarez Carlos, Suárez Karla, Juárez Carmen, Campos Erika, García Verónica, Ruiz Víctor, Soto Claudia.

VI Mexican Congress of Toxicology (Mexico, Federal District, July, 2006). The concentration and the type of selenium in diet have an influence on metabolism of lipids and glucose in mice. Eliud A. García Montalvo, Pedro L. Flores, Olga L. Valenzuela, Claudia A. Soto, Luz Ma. Del Razo.

XLI National Congress of Chemistry of the Mexican Chemical Community (Mexico, Federal District, September, 2006). Relay synthesis and lipoprotective effect *in vivo* of 3β-acetoxi-11α, 12α-epxy-oleanan-28, 13β-olide isolated of *Eysenhardtia platycarpa.* José Manuel Narváez-Mastache, Claudia Soto and Guillermo Delgado.

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Pharmaceutical compound containing Silymarin and Carbopol, production method thereof and use of same as a regenerator of tissue and pancreatic cells with endogenous secretion damaged by diabetes mellitus.

European Patent No. EP 1 576 961 B1 (claims same priority as US Patent Application No. 10/538,277)
Pharmaceutical compound containing Silymarin and Carbopol, production method thereof and use of same as a regenerator of tissue and pancreatic cells with endogenous secretion damaged by diabetes mellitus.

PROJECT SPONSORED BY CONACYT

Effect of Silymarin flavonoide in the regeneration of β-pancreatic cells in diabetes mellitus induced by alloxan (SEP-CONACYT 44614-Q).

OTHER ACADEMICAL ACTIVITIES

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Examiner of Mexican Magazine of Pharmaceutical Sciences.

ACADEMICAL AWARDS

Member of National System of Researchers: candidate of 1993 to 1997, researcher level I of 1997 to 2000 and 2003 to 2009.

1st. Place of Inventor and Innovator Women granted by the National Institute of Women, 2008.

"Iris Estrada" Prize issue 2008 granted by National Institute of Women, 2008.

1st. Place of Medical Research "Jorge Rosenkranz" 2004 granted by Roche Laboratories, 2004.

Honorable Mention in Annual Prize of National Chamber of Pharmaceutical Industry, 2003.

First place in gathering: "The best experience of Social Service in Metropolitan Autonomous University-Xochimilco, 2002 (Social service of student Héctor Barrón Cuevas).

National Prize to Social Service granted by the Department for Social Development 2002 (Social Service of student Héctor Barrón Cuevas).

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Silymarin induces recovery of pancreatic function after alloxan damage in rats

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Abstract

Alloxan has been widely used to produce experimental diabetes mellitus syndrome. This compound causes necrosis of pancreatic β-cells and, as is well known, induces oxidant free radicals which play a relevant role in the etiology and pathogenesis of both experimental and human diabetes mellitus. Previously we have reported hypoglycemic and antilipoperoxidative actions of silymarin in serum and pancreatic tissue respectively. The aim of this study was to test whether silymarin could reduce the hyperglycemia and revert the pancreatic damage in alloxan treated rats, tested with silymarin in two protocols: using both compounds simultaneously for four or eight doses, or using the compound 20 days after alloxan administration for 9 weeks. Serum glucose and insulin were determined, and pancreatic fragments were used for histology and insulin immunohistochemistry. Pancreatic islets were isolated to assess insulin and Pdx1 mRNA expression by RT-PCR.

Our results showed that 72 hours after alloxan administration, serum glucose increased and serum insulin decreased significantly, whereas pancreatic tissue presented morphological abnormalities such as islet shrinkage, necrotic areas, loss of cell organization, widespread lipoid deposits throughout the exocrine tissue, and loss of beta cells, but insulin and glucagon immunoreactivity was scattered if any. In contrast the pancreatic tissue and both

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insulin and glucose serum levels of rats treated with silymarin were similar to those of control animals. In addition, insulin and glucagon immunoreactive cells patterns in Langerhans islets were also normal, and normal insulin and Pdx1 mRNA expression patterns were detected during pancreatic recovery in Langerhans islets. The overall results suggest that silymarin induces pancreatic function recovery demonstrated by insulin and glucagon expression protein and normoglycemia after alloxan pancreatic damage in rats.

Keywords: Insulin; Glucagon; Free radicals; Diabetes mellitus; Hypoglucemiant action; Langerhans islets; Hyperglycemia

Introduction

Alloxan causes severe necrosis of pancreatic β-cells (Dunn et al., 1943), with the consequent lack of insulin secretion. For this reason it has been widely used to induce experimental diabetes mellitus (DM), and many studies have been performed using this model to explore pancreatic damage. It has been suggested that alloxan induces the production of H_2O_2 and of some free radicals such as $O_2^{\cdot}$ and $^{\cdot}OH$ which produce cellular damage followed by cell death. Therefore, the above model was considered adequate for the study of a pathology, such as diabetes mellitus (Heikkila et al., 1976; Winterbourn and Munday, 1989; Soto et al., 1994), in which free radicals might have a central role.

Silymarin is a flavonoid obtained from the milk thistle *Silybum marianum* and its pharmacological profile has been previously described. Its protective effects against the oxidative peroxidation in several experimental models and in human hepatic damage has been previously demonstrated. At pharmacological and clinical levels, silymarin has been extensively used for the treatment of liver damage due to cirrhosis secondary to alcohol abuse as well as other liver pathologies (Wellington and Harvis, 2001; Luper, 1998; Valenzuela and Garrido, 1994). In both cases, silymarin functioning as a free radical scavenger, increasing reduced glutathione (GSH) which functions as a detoxificant of intermediary oxygen reactive products of lipoperoxidation (Valenzuela et al., 1989). Silymarin's mode of action might also be related to its capacity to inhibit enzymatic peroxidation through the lipoxygenase pathway, avoiding leukotriene synthesis (Alarcón de la Lastra et al., 1992). In a previous study (Soto et al., 1998) we used silymarin in pancreas from alloxan treated rats, and found that it was able to prevent a rise in both plasma glucose and pancreatic lipid peroxidation in the hyperglycemic rats. Thus we suggested that the protective effect of silymarin could be due either to its antioxidant properties or to an increase of plasma and pancreatic glutathione concentrations, or both. In addition we recently reported that silymarin induced an increase in the pancreatic activity of antioxidant enzymes: glutathione peroxidase, superoxide dismutase and catalase (Soto et al., 2003).

There has been strong support for the suggestion that reactive oxygen species play a relevant role in the etiology and pathogenesis of DM and its long-term effects (Paolisso et al., 1993; Halliwell, 1994). Nishikawa et al. (1999) found that in hyperglycemic states there was a marked increase in free radicals raised from the electronic chain. In addition, glucose can undergo a process of auto oxidation which is catalyzed by trace amounts of transition metals, generating superoxide and hydroxyl radicals, hydrogen peroxide, and reactive ketoaldehydes (Hunt and Dean, 1998). Reduced glutathione constitutes one of the more important protection mechanisms against free radicals such as superoxide ($O_2^{\cdot}$) and hydroxyl ($^{\cdot}OH$) radicals, so the aim of this study was to analyze whether the protective effect of silymarin may participate in the clinical recovery of alloxan-induced diabetes mellitus in rats.

Materials and methods

Silymarin was kindly supplied by Altana Pharma Laboratories (México). All the reagents were of analytical grade, obtained from Sigma Chemical Co (St. Louis MO), Merck and J.T. Baker (México).

The experiments reported in this study were carried out following the guidelines stated in "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and in the "Mexican Law for Animals Protection".

Animal treatments

Male Wistar rats (180-220g body weight, b. wt.) were obtained from our animal facility. They were fed with Purina standard chow, and maintained at 20–22°C. The animals were divided into the following groups: 1) control group without any drug treatment (n = 24), which received vehicles used for silymarin (carbopol, 0.5%, orally) and for alloxan (isotonic saline solution, subcutaneously). Six rats were sacrificed in 0, 3, 5 and 30 days after vehicle administration. 2) Silymarin group, which received four oral doses (200 mg /kg body weight each, n = 18) at 0, 6, 24 and 48 hours. They were sacrificed at 3, 5 and 30 days after the first dose of silymarin. 3) Alloxan group (n = 18), which received a single subcutaneous dose (150 mg/kg b. wt.). Six rats were sacrificed with the same schedule as group 2. 4) Silymarin plus alloxan group (n = 18), which received silymarin with the same doses and schedule as group 2, and one subcutaneous dose of alloxan (as the group 3) 60 min. after the first dose of silymarin. The animals were sacrificed using the same schedule as groups 2 and 3. 5) Silymarin plus alloxan group (n = 6), which was treated like group 4, but these animals received silymarin for seven days (one dose each 24 hours after the third dose). They were sacrificed in 30 days after the first dose of silymarin. 6) Diabetic group treated with silymarin. These animals received a single subcutaneous dose of alloxan (150 mg/kg b. wt.). After 20 days one oral dose of silymarin (200 mg/kg b. wt.) was administered each 24 hours for nine weeks (n = 21). 7) Control of group 6. This group only received the vehicles of alloxan and silymarin using the same schedule of the group 6 (n = 6). 8) These animals were treated with silymarin as the group 6 but alloxan was not administered. 9) This group only received one dose of alloxan and twenty days after the animals were administered with 1 ml of carbopol (vehicle of silymarin) for nine weeks. Animals of the groups 6 to 9 were sacrificed at the end of treatments.

Serum glucose measurement

Serum glucose was measured in 50 μl of serum rat using the orto-toluidine method (Baner, 1985).

Serum insulin content

The serum insulin concentrations were measured with High Rate Insulin ELISA (Mercodia, Uppsala, Sweden) according to the manufacturer's protocol.

Pancreatic histological analysis

Fragments of the pancreatic tail were fixed in 4% paraformaldehyde in PBS and embedded in paraffin. Ten μm-thick sections were obtained, deparaffinized and stained with hematoxylin and eosin.

Sections were analyzed with 10× and 25× objectives on an optical Leitz Wetzlar Microscope (Heidelberg, Germany) adapted to using a Wild Photomat MPS 55 photographic camera.

Immunohistochemistry and confocal microscopy

Immunohistochemistry was carried out using primary monoclonal antibodies (mAb) anti-insulin, a guinea pig IgG (Zymed Labs, Inc, San Francisco, CA), anti-glucagon, and a mouse IgG (Sigma, San Louis, MO). Prior to immunolabelling, deparaffinized 10 μm-thick pancreatic sections were incubated in 0.2% IgG-free albumin in phosphate buffered saline (PBS) for 30 min. Sections were then double labelled with mAbs anti-insulin (1:100 dilution) and anti-glucagon (1:80 dilution), overnight at 4°C. Fluorescein isothiocyanate-tagged (FITC) rabbit anti-guinea pig IgG (1:60 dilution) (Zymed Labs, Inc, San Francisco, CA) and rhodamine isothiocyanate (RITC)-tagged goat- anti-mouse IgG (1:60 dilution) (Pierce, Rockford), for 1 hr at room temperature. Then, sections were mounted in anti-quenching media (Vectashield, Vector Labs., Burlingame, CA). Double-immunolabelled sections were viewed with a 60× (N.A. 1.4) oil immersion objective on an epi-fluorescence Nikon microscope with attached confocal system (Bio-Rad MRC 600, Watford, UK). From each area, 8–10 optical Z-sections (each 1.0 μm) were scanned using the dual channel imaging system of the laser confocal microscope. When both channels were merged, the correspondent pseudocolors green for FITC and red for RITC were displayed at the monitor screen.

Isolation of Langerhans islets from total pancreas

Pancreatic islets were isolated as described previously by Hiriart and Ramírez-Medeles (1991), with minor modifications. Briefly, pancreatic islets from the splenic portion of the pancreas were separated from the acinar tissue by collagenase digestion and a Ficoll gradient centrifugation; clean islets were then hand-picked and used after repeated washes with Hank's balanced salt solution.

RNA Isolation and RT-PCR for insulin, and Pdx1 detection

The total islet RNA was extracted from pancreatic islets of rats under the different experimental conditions described above. The TRIzol reagent (Invitrogen, Carlsbad, CA) was used following the manufacturer instructions to extracted islet RNA. Islets were lysed in a culture dish by adding 1 ml of TRIzol per 400 islets. Total RNA (400 ng) was reversed transcribed following the supplier's recommended protocol (Invitrogen, Carlsbad, CA).

RT-PCR was carried out with 400 ng of total RNA for mRNA gene detection in islets. A parallel reaction was carried out in the same mRNA sample using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) constitutive gene for quantitative purposes.

All oligonucleotide primers were synthesized and used to prime the amplification of the cDNA template, based on the previous published sequences of insulin, Pdx1, and GAPDH. The sequences used are described respectively: 5′ AAGAGCCATCAGCAAGC-3′ sequence for the sense (5′) primer and the 5′-GAGCAGATGCTGGTGCAGC-3′ for the antisense (3′) primer for insulin. The 5′-GCTCACCTCCACCACCACCTT-3′ sequence for the sense (5′) primer and the 5′-GCAGTACGG-GTCCTCTTGTTTTCC-3′ for the antisense (3′) primer for Pdx1 and, the 5′-GCCCCCATGTTTGTGAT-3′ sequence for the sense (5′) primer and the 5′-GCCCCAGCATCAAAGGT-3′ for the antisense (3′)

primer for GAPDH mRNA amplification. Twenty-five cycles of amplification were performed with an annealing temperature of 59°C for insulin and GAPDH and 56°C for Pdx1. Reaction products were sequenced and proved to have a 100% identity with the sequence reported for the insulin, Pdx1, and GAPDH genes (Tso et al., 1985). PCR amplification conditions and analysis were as follows: 5 min at 94°C followed by 25 cycles of 94°C, 56°C, and 72°C 1 min each step. The amplified material was visualized by ethidium bromide staining, following 2% agarose gel and densitometric analysis.

Statistical methods

ANOVA followed by the Tukey and Dunnet test was used to compare the values of the experimental groups at different periods against the control group at the corresponding periods (SPSS, Chicago, Ill. USA). A difference was considered significant when $p<0.05$.

Results

Silymarin induces normal glucose levels in alloxan damaged pancreas

The concentration of serum glucose in normal rats was 6.18 ± 0.212 mmol/L. At 3 days after alloxan administration this value was increased to 40.9 ± 2.76 reaching 42.42 ± 1.46 mmol/L at day 5. After 30 days of alloxan administration the values of serum glucose were maintained at similar levels (Table 1).

The group of rats that were simultaneously treated with silymarin and alloxan did not show an increase in serum glucose levels at three (8.55 ± 0.8 mmol/L) and at five days (6.99 ± 1.42 mmol/L) after treatment. Nevertheless at day 30 these values were not different from the group treated only with alloxan. In order to determine if a longer treatment with silymarin may induce a more sustained effect, we treated a group of animals for seven days (see materials and methods). Importantly, this group of animals presented similar glucose serum values to those of control groups (Table 1).

In order to investigate the effect of silymarin on the increased serum glucose levels induced after 20 days of alloxan treatment, silymarin was administered from day twenty after alloxan and continued for 9 weeks. Glucose was measured each week after alloxan administration and during the period of the

Table 1

TREATMENT	SERUM GLUCOSE mmol/L	SERUM INSULIN ng/ml
Initial control	6.18 ± 0.912	1.0 ± 0.05
Control (30 days)	6.8 ± 0.88	0.92 ± 0.02
Alloxan (3 days)	40.9 ± 2.760***	0.08 ± 0.006***
Alloxan (5 days)	42.42 ± 1.46 ***	0.1 ± 0.001***
Alloxan (30 days)	37.62 ± 3.03***	0.143 ± 0.07***
Alloxan + Silymarin (four doses) (3 days)	8.55 ± 0.80	0.85 ± 0.020
Alloxan + Silymarin (four doses) (5 days)	6.99 ± 1.42	0.95 ± 0.03
Alloxan + Silymarin (four doses) (30 days)	29.083 ± 2.85***	0.20 ± 0.04***
Alloxan + Silymarin (eight doses) (30 days)	8.7 ± 1.63	0.88 ± 0.07
Silymarin (3 days)	7.02 ± 0.6	1.06 ± 0.036
Silymarin (5 days)	6.58 ± 0.17	1.1 ± 0.050
Silymarin (eight doses) (30 days)	7.16 ± 0.66	1.2 ± 0.75

treatment with silymarin. As it can be observed in Fig. 1, serum glucose levels significantly decreased starting from the first week after its administration and reaching the values of the healthy rats control group, nine weeks after silymarin treatment. This recovery effect was not observed in the alloxan treated control group (Fig. 1). Silymarin by itself or control vehicles did not change serum glucose control values (Table 1, Fig. 1).

Silymarin produces a recovery of insulin serum levels in alloxan pancreatic damage in rats

Serum insulin values of control rats were 1.0 ± 0.05 ng/ml. In contrast, in alloxan-treated rats, serum insulin decreased significantly at 3 days (0.08 ± 0.006 ng/ml) and 5 days (0.1 ± 0.001 ng/ml) after alloxan administration. The value was almost constant until the 30th day after treatment (Table 1).

The serum insulin values found in the rats treated simultaneously with alloxan and silymarin were similar to those found in the control group (control = 1.0 ± .05 ng/ml; day 3 = 0.85 ± 0.02 ng/ml; day 5 = 0.95 ± 0.03 ng/ml). However, the insulin level decreased in day 30 after treatment (Table 1). A longer treatment with silymarin for seven days as described above induced a recovery in insulin serum levels (0.88 ± 0.07 ng/ml) which was within the control values (Table 1).

Twenty days after alloxan administration the serum insulin levels in these rats were significantly lower than in the control animals (0.15 ± 0.04 ng/ml). At this time silymarin treatment was started on daily doses for nine weeks. Insulin levels were detected at the end of the treatment and the serum concentration was within normal group values (Fig. 2). Treatments only with silymarin or vehicles did not change insulin serum levels (Fig. 2).

Silymarin induced pancreas normal histology of alloxan-treated rats

Normal rat pancreas tissue morphology is depicted in Fig. 3A. Its typical septal and intralobular ducts (c) distribution, as well as conjunctive tissue lobules (t) can be observed. Langerhans islets (i) are sparsely found surrounded by the exocrine tissue (a), and normal vessels (v) (Fig. 3A). At 3 days after

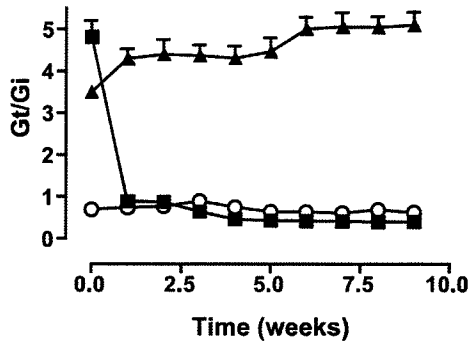

Fig. 1. Serum glucose concentration values of rats treated (■) and untreated (▲) with silymarin after twenty days of alloxan administration with their respective controls (○). Data are expressed as the ratio between serum glucose concentration value at the 20th day after alloxan administration (Gi) to that of further weekly values during nine weeks (Gt). Each point represents the mean ± SEM of 15 animals in the silymarin treated group and 6 animals in the control group. The group of animals treated only with alloxan was initiated with 30 rats but these were dying along the nine weeks. $p \leq 0.001$ between silymarin treated animals (■) and alloxan treated animals (▲) from first week to 9th week. No differences were found between normal and silymarin treated animals.

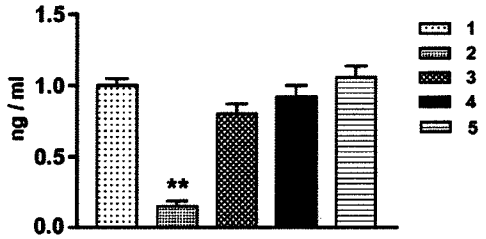

Fig. 2. Serum insulin concentration in rats treated and untreated with silymarin for nine weeks after alloxan administration with their respective controls. Each bar represents the mean value ± SEM of 6 animals for initial control group (1), 6 rats in the group intoxicated with alloxan 20 days after its administration (2), 21 animals in the group of Alloxan and silymarin treated animals(3), and 6 for ending control group (4). **$p < 0.01$ compared with the all other groups.

alloxan administration, the pancreatic tissue from treated rats showed islets shrinkage (i), and lipoid deposits (at) widespread throughout the exocrine tissue (Fig. 3B). All these morphological changes are characteristics of alloxan damaged rat pancreas (1). At the 5th day after alloxan administration no traces of endocrine tissue were found. In addition, other tissue alterations like hyaline degeneration (hd), fibrin deposits (fd), cellular disorder (cd), and necrotic areas (n) were observed (Fig. 3C). Moreover, tissues of rats after 30 days of alloxan administration showed abundance of hemorrhagic areas (hz), lymphocyte infiltration (li), congestive vessels (cv), erythrocyte lysis (el), and islets loss (Fig. 3D).

After one dose of alloxan, rats were simultaneously treated with silymarin for 48 hours. Three days after alloxan administration, no pancreatic tissue morphological difference could be seen as compared with normal non-treated rat controls (Fig. 4A). Similar observations were made after 5 days of alloxan treatment (Fig. 4B). The pancreatic tissue of rats treated with silymarin for seven days as mentioned above (group 5) was analyzed after 30 days. As Fig. 4B shows, this tissue remained unaffected (Fig. 4C). Pancreatic histological examination of rats treated with silymarin for over 9 weeks, starting 20 days after alloxan administration was undistinguishable from normal non-treated rat controls (Fig. 4D).

Confocal microscopy allowed us to assess insulin and glucagon immunolabelling in the endocrine islets (Fig. 5). In A) structures showed characteristic insulin β-cells surrounded by a glucagon immunoreactive layer (head arrows and complete arrows respectively). In B) the confocal analysis to detect insulin and glucagon after 3 days of alloxan treatment did not show any labelling. Confocal analysis of a pancreas after 5 days of alloxan treatment showed scattered if any islets displaying a poor and diffuse double labelling (Fig. 5C). In Fig. 5D, 30 days after alloxan treatment, the aspect of islets in confocal analysis was similar to those of 5 days.

In Fig. 6 we have shown that the confocal analysis of pancreatic tissue of rats treated with silymarin confirmed that the Langerhans islets appeared to be normal in this group of rats. A) 3 days after silymarin treatment, B) 5 days, C) seven days, and D) 9 weeks with silymarin treatment of rats previously treated with one dose of alloxan.

Insulin and Pdx1 gene expression after pancreatic recovery by silymarin

In order to investigate at the molecular level the function of β pancreatic cells of the recovered pancreatic tissue (animals treated with silymarin for 9 weeks after alloxan administration) we isolated the pancreatic islets of this group of animals to obtain the RNA. Total RNA was used for RT-PCR amplification for insulin and Pdx1 genes. The PCR products of these genes were sequenced to determine

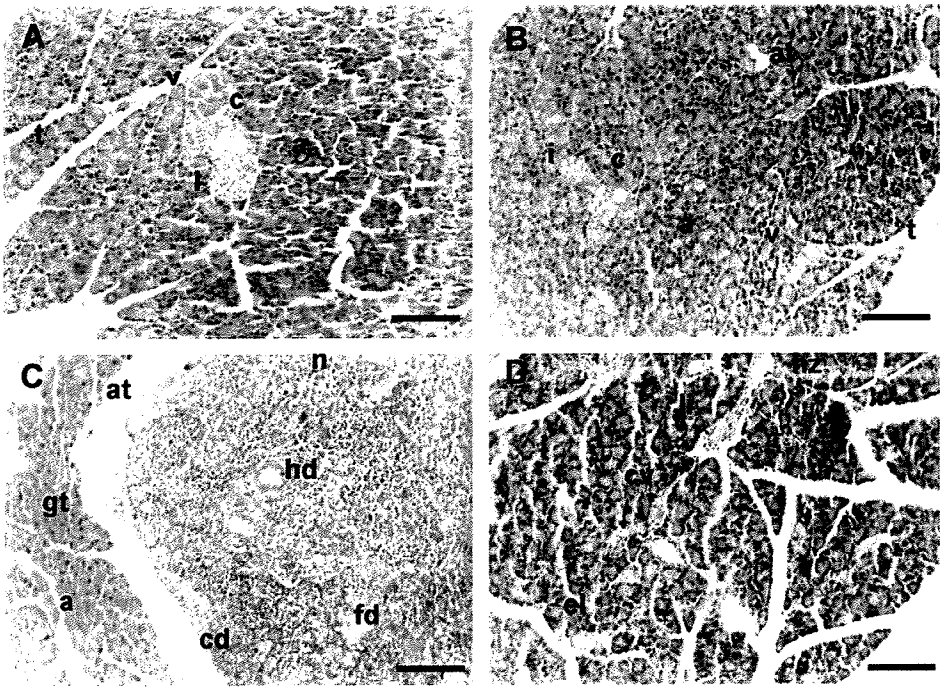

Fig. 3.

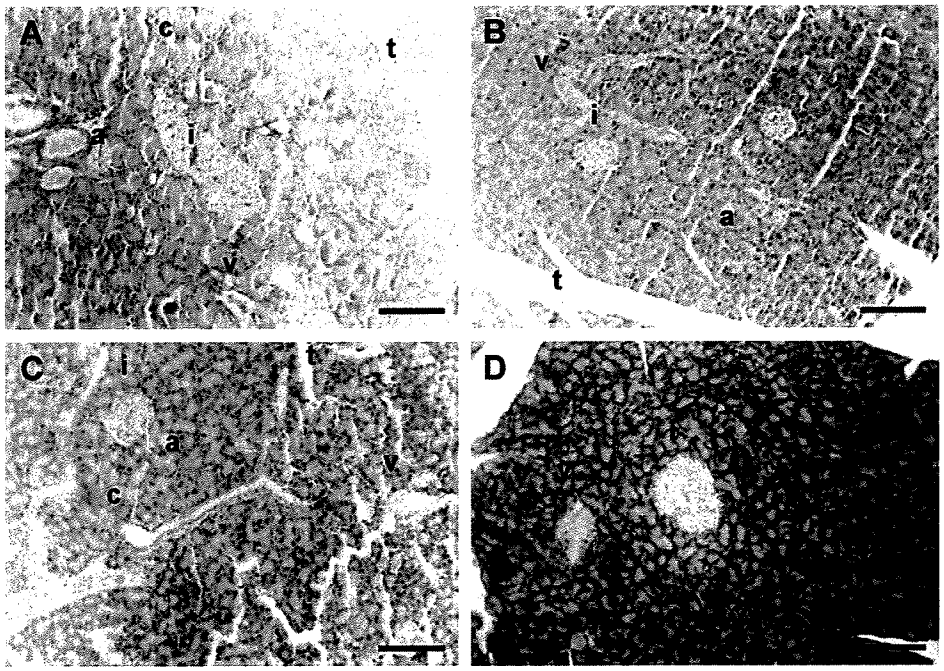

Fig. 4.

homology with insulin and Pdx1 genes respectively (data not shown). It was not possible to isolate pancreatic islets of the groups treated only with alloxan by the lack of these structures in the pancreatic tissue of these animals (see Figs. 4 and 6). Similar insulin and Pdx1 mRNA content was observed between normal and alloxan followed by silymarin treated animals (Fig. 7).

Assessment of body weight

The initial weight of rats was 180 to 220 g. This value increased to 250 ± 20 g after 5 days in the control group. In contrast, 3 days after alloxan administration the weight of rats was not different from the initial value, but at day 5 this value decreased significantly to reach 150 ± 10 g. Simultaneous treatment with silymarin prevented the loss of body weight of these animals. Twenty days after one dose of alloxan administration the weight registered was 180.0 ± 20.0 g. The animals were weighted weekly for 9 weeks (Fig. 8). The variability in body weight between animals is presented as the ratio between the initial weight (Wi) and weekly weight (Wt). Control animals increased their weight approximately 50% of their initial weight (Fig 8). On the other hand, alloxan-treated animals gradually showed a 50% loss of weight with respect to the controls (Fig. 8).

As opposed to results observed in alloxan-treated rats, the group of animals treated with silymarin twenty days after alloxan administration increased their weight in the same way as those of the control group, presenting a 50% weight gain (Fig. 8).

Animal survival

Silymarin increased survival after three days of administration. It is noteworthy that in the group of animals treated with silymarin for nine weeks after 20 days of alloxan administration, the survival rate was dramatically increased. (Table 2).

Discussion

Previously we had demonstrated that silymarin prevented alloxan-induced diabetes mellitus in rats. This effect was attributed to silymarin antioxidant properties (Soto et al., 1998). The present study provides evidences that in alloxan-induced diabetes mellitus affected rats, silymarin recovered the endocrine

Fig. 3. Microphotographs of pancreatic tissue examined by routine hematoxylin-eosin of alloxan treated animals. A) control, B) alloxan after 3 days of administration, C) alloxan after 5 days of administration, D) alloxan after 30 days of administration. i) Langerhans islet, a) acini, v) vessel, c) conduct, t). conjunctive tissue, hz) hemorrhagic zones, n) necrosis, at) adipose tissue, gt). glandular tissue, cd). cellular disorder, fd). fibrin deposit, hd). hyaline degeneration, li). lymphocyte infiltration, el). erythrocyte lysis. Scale bar = 100 μm.

Fig. 4. Microphotographs of pancreatic tissue examined by routine hematoxylin-eosin of alloxan plus silymarin treated animals. A) Simultaneous treatment with alloxan (one dose) plus silymarin (four doses) 3 days after the first dose of silymarin administration. B) Simultaneous treatment with alloxan (one dose) plus silymarin (four doses) 5 days after the first dose of silymarin administration. C) Simultaneous treatment of alloxan (one dose) plus silymarin (eight doses, one daily dose after the second administration) 30 days after the first dose of silymarin administration. D) Silymarin treatment for nine weeks after 20 days of alloxan administration. i) Langerhans islet, a) acini, v) vessel, c) conduct, t) conjunctive tissue. Scale Bar = 100 μm.

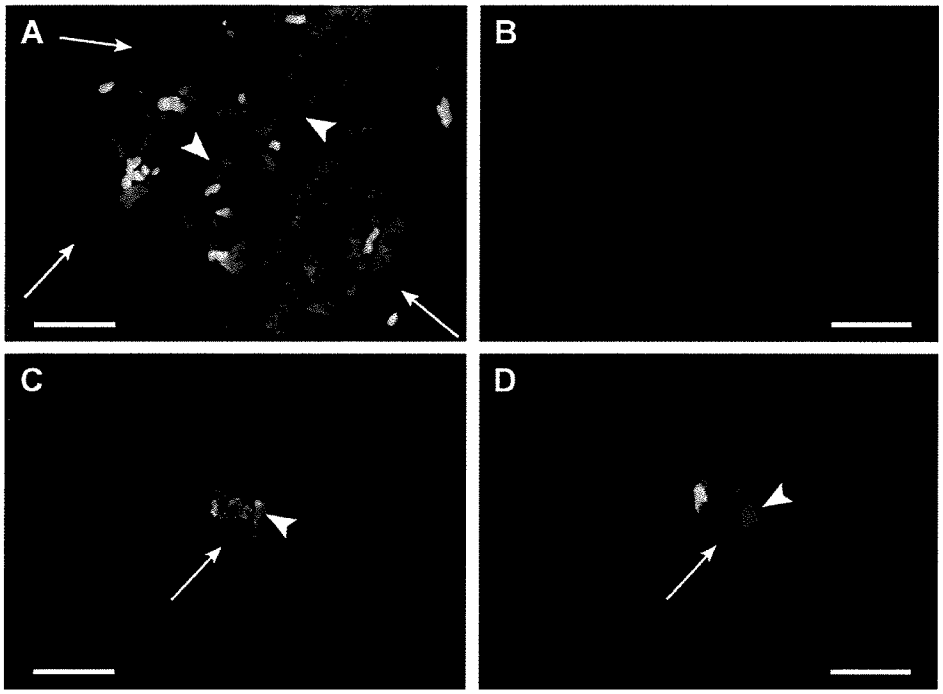

Fig. 5.

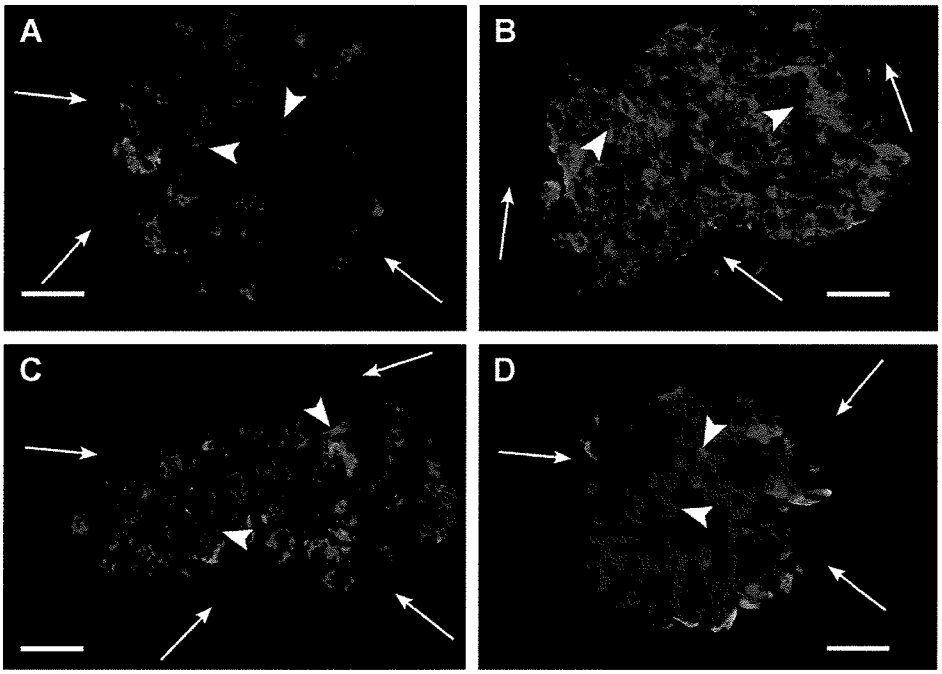

Fig. 6.

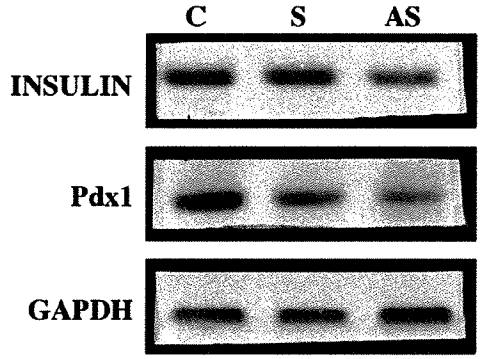

Fig. 7. Insulin and Pdx1 expression. Insulin and Pdx1 mRNA expression detected by RT-PCR amplification. C) control group, S) silymarin treated group, AS) group of animals treated daily with silymarin for nine weeks 20 days after one dose of alloxan.

pancreatic tissue, at both structural and functional levels. To our knowledge this is the first report in a model of experimental pancreatic damage that demonstrates that silymarin may posses these properties.

The induction of experimental hyperglycemia by alloxan in rats was used as a model in the present study. The treatment of hyperglycemic rats with silymarin simultaneously with alloxan, or nine weeks after this toxic substance, induced a restoration of normal glucose and insulin levels to those observed in normal control animals (Table 1). Also, at the histological level, the pancreatic tissue, including both Langerhans islets and exocrine areas, was similar to normal controls (Figs. 1–3). By using confocal microscopy analysis we observed an increase in insulin and glucagon immunoreactivity during the treatment with silymarin. These results are consistent with and support our data of insulin serum levels detected in silymarin treated rats (Fig. 4).

In other hand, the weight, size, and physical characteristics of rats during recovery after silymarin treatment was similar to normal control animals. Taken together, this data strongly suggests that silymarin plays a key molecular role in the structural and functional recovery of the pancreas in alloxan induced degeneration of this tissue in rats.

Alloxan experimental models of pancreatic damage have been demonstrated with structural and functional alterations similar to that observed in our model, such as disorganization of pancreatic architecture, and depletion of insulin producing cells (Davidson et al., 1989; Waguri et al., 1997). Indeed, extensive damage of pancreatic tissue may be detected 24h after alloxan administration. Waguri et al. (1997) carried out studies on β-pancreatic cell regeneration from 1 day to 48 weeks after alloxan administration in a mouse model. They had evidence of small β-pancreatic cell regeneration of almost

Fig. 5. Double labelling with anti-insulin (green channel) and anti-glucagon (red channel) mAbs in rat endocrine pancreatic tissue (Langerhans islet) of animals treated with alloxan, A) control, B) alloxan after 3 days of administration, C) alloxan after 5 days of administration, D) alloxan after 30 days of administration. Arrow heads indicate insulin and complete arrows indicate glucagon. Scale Bar = 20 μm.

Fig. 6. Double labelling with anti-insulin (green channel) and anti-glucagon (red channel) mAbs in rat endocrine pancreatic tissue (Langerhans islet) of animals treated with alloxan plus silymarin. A) Simultaneous treatment with alloxan (one dose) plus silymarin (four doses) 3 days after the first dose of silymarin administration, B) simultaneous treatment with alloxan (one dose) plus silymarin (four doses) 5 days after the first dose of silymarin administration, C) simultaneous treatment with alloxan (one dose) plus silymarin (eight doses, one daily dose after the second administration) 30 days after the first dose of silymarin administration, D) silymarin treatment for nine weeks after 20 days of alloxan administration. Arrow heads indicate insulin and complete arrows indicate glucagon. Scale Bar = 20 μm.

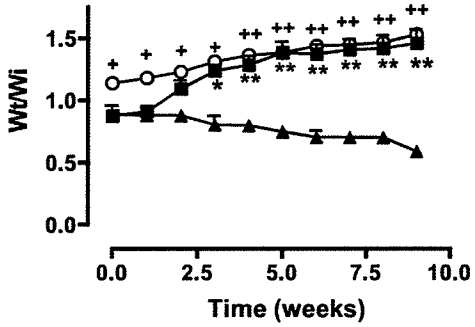

Fig. 8. Body weight values of rats treated (■) and untreated (▲) with silymarin after twenty days of alloxan administration with their respective controls (O). Data are expressed as the ratio between the weight value at the 20th day after Alloxan administration (Wi) to that of further weekly values during nine weeks (Wt). Each point represents the mean ± SEM of 15 animals in the silymarin treated group and 6 animals in the control group. The group of animals treated only with alloxan group was initiated with 30 rats, but animals were dying along the nine weeks. $^{+}p < 0.05$, $^{++}p < 0.01$ with respect to alloxan treated group.*$p < 0.05$, **$p < 0.01$ with respect to alloxan treated group.

2.5% at 48 weeks. In the present study, the pancreas of rats treated with silymarin presented evidence of β-cell recovery from the third day when silymarin was administered on the first day of alloxan treatment. At 9 weeks we observed a similar histomorphology of the pancreatic tissue as in normal controls (Figs. 4–5), thus indicating that we had reached an increased recovery value which was significantly higher that previously reported in similar animal models, supported by the fact that silymarin restored the structure and function of the rat alloxan treated pancreas.

It has been previously reported that chronic exposure of culture-grown HIT-T15 cells to supraphysiological concentration of glucose cause gradual loss of both insulin and Pdx1 gene expression. The mechanism of this adverse event involves the loss of mRNA and protein levels of pancreas duodenum homeobox-1 (Pdx1), a critical regulator of insulin promoter activity. In that animal model the loss of islet DNA binding activity of Pdx1 has been observed, as well as insulin gene expression related with hyperglycemic state (Robertson et al., 1992; Harmon et al., 1999). Interestingly, in the present work we detected insulin mRNA expression together with normoglycemia and Pdx1

Table 2

TREATMENT	SURVIVAL (%)
Controls	100
Alloxan (3 days)	59 ± 3
Alloxan (5 days)	50 ± 5
Alloxan (30 days)	30 ± 5
Alloxan + Silymarin (four doses) 3 days	100
Alloxan + Silymarin (four doses) 5 days	100
Alloxan + Silymarin (four doses) 30 days	70 ± 2
Alloxan + Silymarin (eight doses) 30 days	100
Alloxan (12 weeks)	7 ± 5
Alloxan (20 days) followed + Silymarin (9 weeks)	86.7 ± 1
Silymarin	100

expression in silymarin-alloxan treated animals (Fig. 5), thus suggesting that a normal Pdx1 regulation of insulin gene was re-established in treated animals.

It has previously been demonstrated that antioxidants maintain insulin and their mRNA levels, and also the expression of Pdx1 was more evident in the nuclei of β-cells after the antioxidant treatment (Kaneto et al., 1999).

The pharmacodynamic profile of silymarin has been well studied (Wellington and Harvis, 2001). At the pharmacological and clinical levels, silymarin has been extensively used for the treatment of liver damage due to cirrhosis secundary to alcohol abuse as well as other liver pathologies (Luper, 1998; Valenzuela and Garrido, 1994; Flora et al., 1998). The hepatoprotective mechanisms of silymarin involve a number of different biochemical events. It has been shown that silymarin increases the synthesis of ribosomal RNA (rRNA) species through stimulation of polymerase I and rRNA transcription (Utrilla, 1996). This stimulation may enable hepatic cells to counteract the functional impairment of transporters and enzymes that occurs during liver damage. However, the better known mechanism of hepatic protection of silymarin is due to its action on oxidative stress (Morazzoni and Bombardelli, 1995). Indeed, the ability of silymarin to protect against oxidative stress-induced hepatocellular damage (such as lipid peroxidation of membranes and subsequent membrane degradation) is associated with these free radical scavenging properties and its ability to enhance endogenous antioxidant defences, such as those mediated by superoxide dismutase (SOD) or the glutathione system. In this regard, glutathione (GSH) has been implicated in a variety of cellular processes, including detoxification of electrophillic substances and peroxides, control of enzyme activity, and regulation of cell cycle (Shafer and Buettner, 2001). It has been proposed that oxidative stress contributes to the development of diabetic complications. Antioxidants such as ascorbic acid, vitamin E and glutathione are all decreased in both experimental and human diabetes mellitus (Som et al., 1981; Gokkusu et al., 2001). Increased levels of plasma lipid peroxidation products are also found (Yoshida et al., 1995). The demonstration that glucose can be oxidized catalytically by trace amounts of transition metals generating free radicals, hydrogen peroxide, and reactive ketoaldehydes, is consistent with this hypothesis.

The importance of human diabetes mellitus as a world health problem is due to the fact that at least 150 million people are affected, thus the necessity to seek new drugs. The existent ones only favor insulin release or control blood glucose level but do not recover the endocrine pancreatic function. The data presented in this study suggest that silymarin represents a new possibility in the treatment of diabetes mellitus, not only for the enhanced insulin levels but also for pancreatic function recovery. In addition to our findings, it has been reported that silymarin did not produce any collateral adverse effect. Although more studies are required to demonstrate its beneficial properties in human diabetes mellitus.

In conclusion, the evidences presented in this study support that silymarin not only has a protective effect on rat alloxan-induced diabetes mellitus but also induces pancreas recovery.

Acknowledgments

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CHAPTER 60

INSULIN, ORAL HYPOGLYCEMIC AGENTS, AND THE PHARMACOLOGY OF THE ENDOCRINE PANCREAS

Stephen N. Davis

INSULIN

In recent years, developed nations have witnessed an explosive increase in the prevalence of diabetes mellitus (DM) predominantly related to lifestyle changes and the resulting surge in obesity. The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease, and blindness, pose an enormous burden on patients with diabetes mellitus and on the public health system. Improvements in our understanding of the pathogenesis of diabetes and its complications and in the therapy and prevention of diabetes are critical to meeting this health care challenge.

History. Few events in the history of medicine are more dramatic than the discovery of insulin. Although the discovery is appropriately attributed to Banting and Best, others provided important observations and techniques that made it possible. In 1869, a German medical student, Paul Langerhans, noted that the pancreas contains two distinct groups of cells—the acinar cells, which secrete digestive enzymes, and cells that are clustered in islands, or islets, which he suggested served a second function. Direct evidence for this function came in 1889, when Minkowski and von Mering showed that pancreatectomized dogs exhibit a syndrome similar to diabetes mellitus in humans.

There were numerous attempts to extract the pancreatic substance responsible for regulating blood glucose. In the early 1900s, Gurg Zuelzer, an internist in Berlin, attempted to treat a dying diabetic patient with extracts of pancreas. Although the patient improved temporarily, he sank back into a coma and died when the supply of extract was exhausted. E.L. Scott, a student at the University of Chicago, made another early attempt to isolate an active principle in 1911. Using alcoholic extracts of the pancreas (not so different from those eventually used by Banting and Best), Scott treated several diabetic dogs with encouraging results; however, he lacked clear measures of control of blood glucose concentrations, and his professor considered the experiments inconclusive at best. Between 1916 and 1920, the Romanian physiologist Nicolas Paulesco found that injections of pancreatic extracts reduced urinary sugar and ketones in diabetic dogs. Although he published the results of his experiments, their significance was fully appreciated only years later.

Unaware of much of this work, Frederick Banting, a young Canadian surgeon, convinced J.J.R. Macleod, a professor of physiology in Toronto, to allow him access to a laboratory to search for the antidiabetic principle of the pancreas. Banting assumed that the islets secreted insulin but that the hormone was destroyed by proteolytic digestion prior to or during extraction. Together with Charles Best, a fourth-year medical student, he attempted to overcome the problem by ligating the pancreatic ducts. The acinar tissue degenerated, leaving the islets undisturbed; the remaining tissue then was extracted with ethanol and acid. Banting and Best thus obtained a pancreatic extract that decreased the concentration of blood glucose in diabetic dogs.

The first patient to receive the active extracts prepared by Banting and Best was Leonard Thompson, aged 14. He presented at the Toronto General Hospital with a blood glucose level of 500 mg/dl (28 mM). Despite rigid control of his diet (450 kcal/day), he continued to excrete large quantities of glucose, and without insulin, the most likely outcome would be death after a few months. The administration of Banting and Best's extracts reduced the plasma concentration and urinary excretion of glucose. Daily injections were given. Glucose excretion was reduced from over 100 to as little as 7.5 g/day, and the patient demonstrated marked clinical improvement. Thus replacement therapy with the newly discovered hormone, insulin, had interrupted what was clearly an otherwise fatal metabolic disorder. Banting and Best faced many trials and tribulations during the subsequent year. It was difficult to obtain active extracts reproducibly. This led to a greater involvement of Macleod; Banting also sought help from J.B. Collip, a chemist with expertise in extraction and purification of epinephrine. Stable extracts eventually were

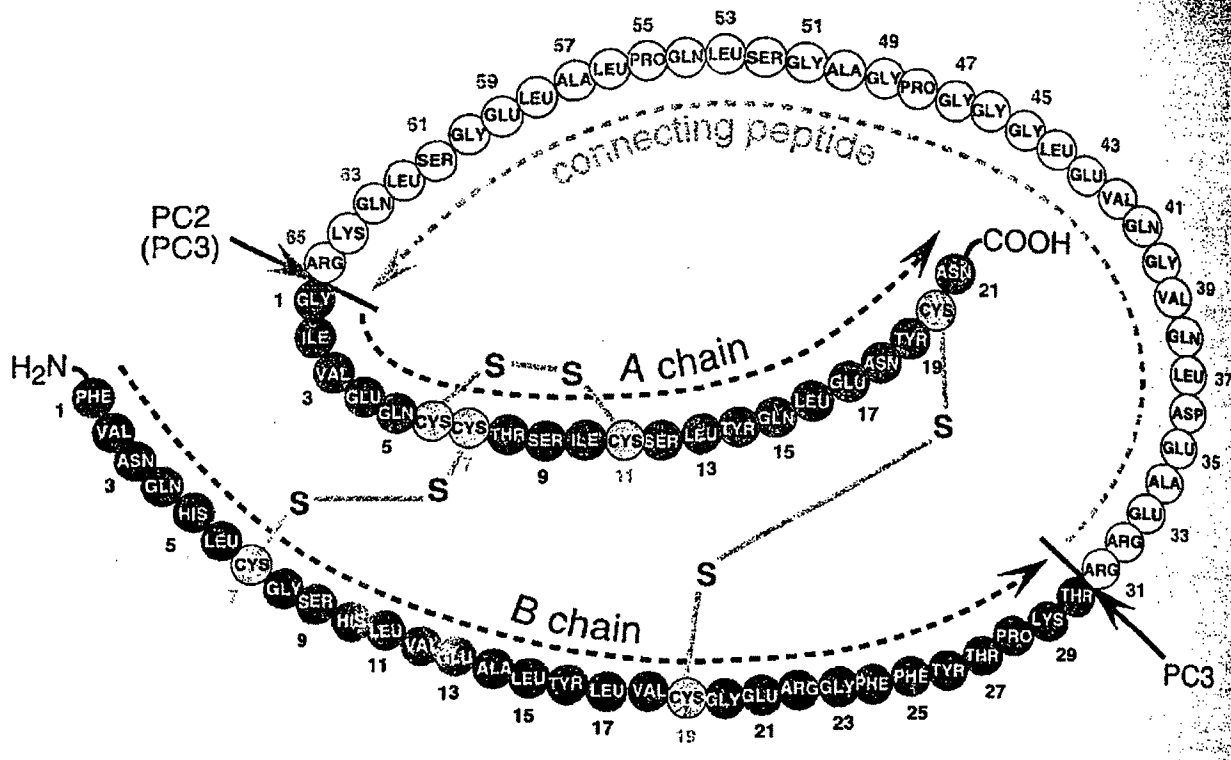

Figure 60–1. Human proinsulin and its conversion to insulin. The amino acid sequence of human proinsulin is shown. By proteolytic cleavage, four basic amino acids (residues 31, 32, 64, and 65) and the connecting peptide are removed, converting proinsulin to insulin. The sites of action of the endopeptidases PC2 and PC3 are shown.

obtained, and patients in many parts of North America soon were being treated with insulin from porcine and bovine sources. Now, as a result of recombinant DNA technology, human insulin is used for therapy.

The Nobel Prize in medicine and physiology was awarded to Banting and Macleod with remarkable rapidity in 1923, and a furor over credit followed immediately. Banting announced that he would share his prize with Best; Macleod did the same with Collip.

Chemistry. Insulin was purified and crystallized by Abel within a few years of its discovery. Sanger established the amino acid sequence of insulin in 1960, the protein was synthesized in 1963, and Hodgkin and coworkers elucidated insulin's three-dimensional structure in 1972. Insulin was the hormone for which Yalow and Berson first developed the radioimmunoassay (Kahn and Roth, 2004).

The β (or B) cells of pancreatic islets synthesize insulin from a single-chain precursor of 110 amino acids termed *preproinsulin.* After translocation through the membrane of the rough endoplasmic reticulum, the 24-amino-acid N-terminal signal peptide of preproinsulin is cleaved rapidly to form proinsulin (Figure 60–1). Thereafter, proinsulin folds, and the disulfide bonds form. During conversion of human proinsulin to insulin, four basic amino acids and the remaining connector or C peptide are removed by proteolysis. This gives rise to the A and B peptide chains of the insulin molecule, which contains one intrasubunit and two intersubunit disulfide bonds. The A chain usually is composed of 21 amino acid residues, and the B chain has 30; the molecular mass is thus about 5734 daltons. Although the amino acid sequence of insulin has been highly conserved in evolution, there are significant variations that account for differences in both biological potency and immunogenicity (De Meyts, 1994). There is a single insulin gene and a single protein product in most species. However, rats and mice have two genes that encode insulin and synthesize two molecules that differ at two amino acid residues in the B chain.

The crystal structure reveals that the two chains of insulin form a highly ordered structure with α-helical regions in each of the chains. The isolated chains of insulin are inactive. In solution, insulin can exist as a monomer, dimer, or hexamer. Two molecules of Zn^{2+} are coordinated in the hexamer, and this form of insulin presumably is stored in the granules of the pancreatic β cell. It is believed that Zn^{2+} has a functional role in the hexamer formation and that this process facilitates the conversion of proinsulin to insulin and storage of the hormone. Traditional insulin is hexameric in most of the highly concentrated preparations used for therapy. When the hormone is absorbed and the concentration falls to physiological levels (nanomolar), the hormone dissociates into monomers, and the monomer is most likely the biologically active form of insulin. Monomeric insulin is now available for therapy.

Substantial information about the structure–activity relationship of insulin has been obtained by study of insulins purified from a wide variety of species and by modification of the molecule. A dozen invariant residues in the A and B chains form a surface that interacts with the insulin receptor (Figure 60–2). These residues

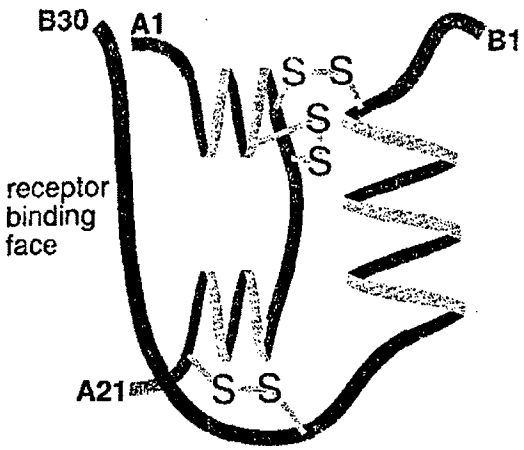

Figure 60–2. *Model of the three-dimensional structure of insulin.* The shaded area indicates the receptor-binding face of the insulin molecule.

Gly^{A1}, Glu^{A4}, Gln^{A5}, Tyr^{A19}, Asn^{A21}, Val^{B12}, Tyr^{B16}, Gly^{B23}, Phe^{B24}, Phe^{B25}, and Tyr^{B26}—overlap with domains that also are involved in insulin dimerization (De Meyts, 1994). The Leu^{A13} and Leu^{B17} residues may form part of a second binding surface. Insulin binds to surfaces located at the N- and C-terminal regions of the α subunit of the receptor, including a cysteine-rich region in the receptor α chain. In most cases, the affinity of insulin for the insulin receptor correlates closely with its potency for eliciting effects on glucose metabolism. Human, bovine, and porcine insulins are equipotent; South American guinea pig insulin is much less potent, whereas certain avian insulins are significantly more so.

Insulin is a member of a family of related peptides termed *insulinlike growth factors* (IGFs). The two IGFs (IGF-1 and IGF-2) have molecular masses of about 7500 daltons and structures that are homologous to that of proinsulin. However, the short equivalents of the C peptide in proinsulin are not removed from the IGFs. In contrast with insulin, the IGFs are produced in many tissues, and they may serve a more important function in the regulation of growth than in the regulation of metabolism. These peptides, particularly IGF-1, are the presumed mediators of the action of growth hormone, and they originally were called *somatomedins*. The uterine hormone *relaxin* also may be a distant relative of this family of polypeptides, although the relaxin receptor clearly is distinct from those for insulin and IGF-1.

The receptors for insulin and IGF-1 are also closely related (Nakae *et al.*, 2001). Thus, insulin can bind to the receptor for IGF-1 with low affinity and *vice versa*. The growth-promoting actions of insulin appear to be mediated in part through the IGF-1 receptor, and there may be discordance between the metabolic potency of an insulin analog and its ability to promote growth. For example, proinsulin has only 2% of the metabolic potency of insulin *in vitro*, but it is half as potent as insulin in stimulating mitogenesis.

Synthesis, Secretion, Distribution, and Degradation of Insulin

Insulin Production. The molecular and cellular events involved in the synthesis, storage, and secretion of insulin by the β cell and ultimate degradation of the hormone by its target tissues have been studied in great detail and have served as a model for study of other cell types in the pancreatic islet. The islet of Langerhans is composed of four types of cells, each of which synthesizes and secretes a distinct polypeptide hormone: insulin in the β (B) cell, *glucagon* in the α (A) cell, *somatostatin* in the δ (D) cell, and pancreatic polypeptide in the PP or F cell. The β cells make up 60% to 80% of the islet and form its central core. The α, δ, and F cells form a discontinuous mantle, one to three cells thick, around this core.

The cells in the islet are connected by tight junctions that allow small molecules to pass and facilitate coordinated control of groups of cells. Arterioles enter the islets and branch into a glomerularlike capillary mass in the β-cell core. Capillaries then pass to the rim of the islet and coalesce into collecting venules. Blood flows in the islet from the β cells to α and δ cells. Thus, the β cell is the primary glucose sensor for the islet, and the other cell types presumably are exposed to particularly high concentrations of insulin.

As noted earlier, insulin is synthesized as a single-chain precursor in which the A and B chains are connected by the C peptide. The initial translation product, preproinsulin, contains a sequence of 24 primarily hydrophobic amino acid residues attached to the N terminus of the B chain. This signal sequence is required for the association and penetration of nascent preproinsulin into the lumen of the rough endoplasmic reticulum. This sequence is cleaved rapidly, and proinsulin is then transported in small vesicles to the Golgi complex. Here, proinsulin is packaged into secretory granules along with the enzyme(s) responsible for its conversion to insulin.

The conversion of proinsulin to insulin begins in the Golgi complex, continues in the secretory granules, and is nearly complete at the time of secretion. Thus, equimolar amounts of C peptide and insulin are released into the circulation. The C peptide has no known biological function but serves as a useful index of insulin secretion in distinguishing between patients with factitious insulin injection and insulin-producing tumors. Small quantities of proinsulin and des-31,32 proinsulin also are released from β cells. This presumably reflects either exocytosis of granules in which the conversion of proinsulin to insulin is not complete or secretion by another pathway. Since the half-life of proinsulin in the circulation is much longer than that of insulin, up to 20% of immunoreactive insulin in plasma is, in reality, proinsulin and intermediates.

Two distinct Ca^{2+}-dependent endopeptidases, which are found in the islet cell granules and in other neuroendocrine cells, are responsible for the conversion of proinsulin to insulin. These endoproteases, PC2 and PC3, have catalytic domains related to that of subtilisin and cleave at Lys–Arg or Arg–Arg sequences (Steiner *et al.*, 1996). PC2 selectively cleaves at the C peptide–A chain junction (Figure 60–1). PC3 preferentially cleaves at the C peptide–B chain junction but has some action at the A chain junction as well. Although there are at least two other members of the family of endoproteases (PC1 and furin), PC2 and PC3 appear to be the enzymes responsible for processing proinsulin to insulin.

Regulation of Insulin Secretion. Insulin secretion is a tightly regulated process designed to provide stable concentrations of glucose in blood during both fasting and feeding. This regulation is achieved by the coordinated interplay of various nutrients, gastrointestinal hormones, pancreatic hormones, and autonomic neurotransmitters. Glu-

cose, amino acids, fatty acids, and ketone bodies promote the secretion of insulin. The islets of Langerhans are richly innervated by both adrenergic and cholinergic nerves. Stimulation of α_2 adrenergic receptors inhibits insulin secretion, whereas β_2 adrenergic receptor agonists and vagal nerve stimulation enhance release. In general, any condition that activates the sympathetic branch of the autonomic nervous system (such as hypoxia, hypoglycemia, exercise, hypothermia, surgery, or severe burns) suppresses the secretion of insulin by stimulation of α_2 adrenergic receptors. Predictably, α_2 adrenergic receptor antagonists increase basal concentrations of insulin in plasma, and β_2 adrenergic receptor antagonists decrease them.

Glucose is the principal stimulus to insulin secretion in human beings and is an essential permissive factor for the actions of many other secretagogues (Matschinsky, 1996). The sugar is more effective in provoking insulin secretion when taken orally than when administered intravenously because the ingestion of glucose (or food) induces the release of gastrointestinal hormones and stimulates vagal activity. Several gastrointestinal hormones promote the secretion of insulin. The most potent of these are gastrointestinal inhibitory peptide (GIP) and glucagonlike peptide 1 (GLP-1). Insulin release also is stimulated by gastrin, secretin, cholecystokinin, vasoactive intestinal peptide, gastrin-releasing peptide, and enteroglucagon.

When evoked by glucose, insulin secretion is biphasic: The first phase reaches a peak after 1 to 2 minutes and is short-lived; the second phase has a delayed onset but a longer duration.

Recent research has provided an outline of how glucose stimulates insulin secretion. Basically, the resting β cell is hyperpolarized, and its depolarization leads to the secretion of insulin. A rising plasma glucose concentration initiates a series of events that leads to depolarization. Glucose enters the β cell by facilitated transport, which is mediated by GLUT2, a specific subtype of glucose transporter, whereupon the sugar is phosphorylated to glucose-6-phosphate (G-6-P) by glucokinase. The increase in oxidizable substrate (glucose and G-6-P) enhances adenosine triphosphate (ATP) production, thereby increasing the ATP–adenosine diphosphate (ADP) ratio and inhibiting an ATP-sensitive K^+ channel. This decrease in K^+ conductance causes E_m to rise, opening a voltage-sensitive Ca^{2+} channel.

Intracellular Ca^{2+} acts as the insulin secretagogue, as it does for the secretion of many vesicular products. The influx of Ca^{2+} also activates several phospholipases, leading to the production of eicosanoids and IP_3 and the mobilization of intracellular Ca^{2+} stores.

The ATP-sensitive K^+ channel in insulin-secreting cells is an octamer composed of four Kir 6.2 and four SUR1 subunits. Both types of subunits contain nucleotide-binding domains; Kir 6.2 appears to mediate the inhibitory response to ATP; SUR1 binds ADP, the channel activator *diazoxide*, and the channel inhibitors (and promoters of insulin secretion) sulfonylureas and meglitinide. Mutations in the channel proteins can lead to altered insulin secretion (*see* Proks *et al.*, 2004).

Elevation of free Ca^{2+} concentrations also occurs in response to stimulation of phospholipase C by acetylcholine and cholecystokinin and by hormones that increase intracellular concentrations of cyclic AMP. In the β cell, G protein–coupled receptors (GPCRs) for glucagon, GIP, and GLP-1 couple to G_s to stimulate adenylyl cyclase; somatostatin and α_2 adrenergic receptor agonists couple to G_i to reduce cellular cyclic adenosine monophosphate (AMP) production.

The hexokinase involved in this process is a specific isoform, glucokinase, whose expression is limited primarily to cells and tissues involved in the regulation of glucose metabolism, such as the liver and pancreatic cells. Its relatively high K_m (10 to 20 mM) gives it an important regulatory role at physiological concentrations of glucose. The capacity of sugars to undergo phosphorylation and subsequent glycolysis correlates closely with their ability to stimulate insulin release. The role of glucokinase as a glucose sensor was inferred from the association of mutations of the glucokinase gene with a form of maturity-onset diabetes of the young (MODY2; *see* below), a rare genetic form of diabetes. These mutations, which compromise the ability of glucokinase to phosphorylate glucose, raise the threshold for glucose-stimulated insulin release.

Most of the nutrients and hormones that stimulate insulin secretion also enhance its biosynthesis. Although there is a close correlation between the two processes, some factors affect one pathway but not the other. For example, lowering extracellular concentrations of Ca^{2+} inhibits secretion of insulin without affecting biosynthesis.

There usually is a reciprocal relationship between the rates of secretion of insulin and glucagon from the pancreatic islet. This reciprocity reflects both the influence of insulin on the α cell and the level of glucose and other substrates (*see* below). In addition, somatostatin, a third islet cell hormone, can modulate the secretion of both hormones (*see* below). Glucagon stimulates the release of somatostatin, which may suppress the secretion of insulin but is not a major physiological influence. Since the blood supply in the islet flows from the β-cell core to the α and δ cells, insulin can inhibit glucagon release in a paracrine manner, but somatostatin must pass through the circulation to reach the α and β cells. Thus, while insulin affects the secretion of glucagon and pancreatic polypeptide, the paracrine of islet somatostatin is not clear.

Distribution and Degradation of Insulin. Insulin circulates in blood as the free monomer, and its volume of distribution approximates the volume of extracellular fluid. Under fasting conditions, the pancreas secretes about 40 μg (1 unit) of insulin per hour into the portal vein to achieve a concentration of insulin in portal blood of 2 to 4 ng/ml (50 to 100 μunits/ml) and in the peripheral circulation of 0.5 ng/ml (12 μunits/ml) or about 0.1 nM. After ingestion of a meal, there is a rapid rise in the concentration of insulin in portal blood, followed by a parallel but smaller rise in the peripheral circulation. A goal of insulin therapy is to mimic this pattern, but this is difficult to achieve with subcutaneous injections.

The half-life of insulin in plasma is about 5 to 6 minutes in normal subjects and patients with uncomplicated diabetes. This value may be increased in diabetics who develop anti-insulin antibodies. The half-life of proinsulin is longer than that of insulin (about 17 minutes), and this protein usually accounts for about 10% of the immunoreactive "insulin" in plasma. In patients with insulinoma, the percentage of proinsulin in the circulation usually is increased and may be as much as 80% of immunoreactivity. Since proinsulin is only about 2% as potent as insulin, the biologically effective concentration of insulin is somewhat lower than estimated by immunoassay. C peptide is secreted in equimolar amounts with insulin; however, its molar concentration in plasma is higher because of its lower hepatic clearance and considerably longer half-life

(about 30 minutes). C peptide serves as a marker for acute insulin secretion.

Degradation of insulin occurs primarily in liver, kidney, and muscle (Duckworth, 1988). About 50% of the insulin that reaches the liver *via* the portal vein is destroyed and never reaches the general circulation. Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules, which also degrade it. Severe impairment of renal function appears to affect the rate of disappearance of circulating insulin to a greater extent than does hepatic disease. Hepatic degradation of insulin operates near its maximal capacity and cannot compensate for diminished renal breakdown of the hormone. Peripheral tissues such as fat also inactivate insulin, but this is of less significance quantitatively.

Proteolytic degradation of insulin in the liver occurs primarily after internalization of the hormone and its receptor and, to a lesser extent, at the cell surface. The primary pathway for internalization is receptor-mediated endocytosis. The complex of insulin and its receptor is internalized into small vesicles termed *endosomes*, where degradation is initiated (Duckworth, 1988). Some insulin also is delivered to lysosomes for degradation.

The extent to which internalized insulin is degraded by the cell varies considerably with the cell type. In hepatocytes, over 50% of the internalized insulin is degraded, whereas most internalized insulin is released intact from endothelial cells. In the latter case, this finding appears to be related to the role of these cells in transcytosis of insulin molecules from the intravascular to the extracellular space. Transcytosis has an important role in the delivery of insulin to its target cells in tissues where endothelial cells form tight junctions, including skeletal muscle and adipose tissue.

Several enzymes have been implicated in insulin degradation. The primary insulin-degrading enzyme is a thiol metalloproteinase. It is localized primarily in hepatocytes, but immunologically related molecules also have been found in muscle, kidney, and brain (Duckworth, 1988). Most insulin-degrading enzyme activity appears to be cytosolic, raising the question of how the internalized vesicular insulin becomes associated with the degrading enzyme, although this activity also has been found in endosomes. A second insulin-degrading enzyme also has been described (Authier *et al.*, 1994), but the relative roles of these enzymes have not been established. Insulin-degrading enzyme also may have a role in the degradation of other hormones, including glucagon.

Cellular Actions of Insulin. Insulin elicits a remarkable array of biological responses. The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle, and fat, but insulin exerts potent regulatory effects on other cell types as well. Insulin is the primary hormone responsible for controlling the uptake, use, and storage of cellular nutrients. Insulin's anabolic actions include the stimulation of intracellular use and storage of glucose, amino acids, and fatty acids, whereas it inhibits catabolic processes such as the breakdown of glycogen, fat, and protein. It accomplishes these general purposes by stimulating the transport of substrates and ions into cells, promoting the translocation of proteins between cellular compartments, activating and inactivating specific enzymes, and changing the amounts of proteins by altering the rates of gene transcription and specific mRNA translation (Figure 60–3).

Some effects of insulin occur within seconds or minutes, including the activation of glucose and ion transport systems, the covalent modification (*i.e.*, phosphorylation or dephosphorylation) of enzymes, and some effects on gene transcription (*i.e.*, inhibition of the phosphoenolpyruvate carboxykinase gene) (O'Brien and Granner, 1996). Other effects, such as those on protein synthesis and gene transcription, may take a few hours. Effects of insulin on cell proliferation and differentiation may take days. It is not clear whether these kinetic differences result from the use of different mechanistic pathways or from the intrinsic kinetics of the various processes.

Regulation of Glucose Transport. Stimulation of glucose transport into muscle and adipose tissue is a crucial component of the physiological response to insulin. Glucose enters cells by facilitated diffusion through one of a family of glucose transporters. Five of these (GLUT1 through GLUT5) are thought to be involved in Na^+-independent facilitated diffusion of glucose into cells (Shepherd and Kahn, 1999). The glucose transporters are integral membrane glycoproteins with molecular masses of about 50,000 daltons, and each has 12 membrane-spanning α-helical domains. Insulin stimulates glucose transport at least in part by promoting translocation of intracellular vesicles that contain the GLUT4 and GLUT1 glucose transporters to the plasma membrane (Figure 60–3). This effect is reversible; the transporters return to the intracellular pool on removal of insulin. Faulty regulation of this process may contribute to the pathophysiology of type 2 DM (Shepherd and Kahn, 1999).

Regulation of Glucose Metabolism. The facilitated diffusion of glucose into cells along a downhill gradient is ensured by glucose phosphorylation. This enzymatic reaction, the conversion of glucose to glucose-6-phosphate (G-6-P), is accomplished by one of a family of hexokinases. Like the glucose transporters described earlier, the four hexokinases (I through IV) are distributed differently in tissues, and two are regulated by insulin. Hexokinase IV, a 50,000-dalton enzyme more commonly known as *glucokinase,* is found in association with GLUT2 in liver and pancreatic β cells. There is one glucokinase gene, but different first exons and promoters are employed in the two tissues (Printz *et al.*, 1993). The liver glucokinase gene is regulated by insulin. Hexokinase II, a 100,000-dalton enzyme, is found in associa-

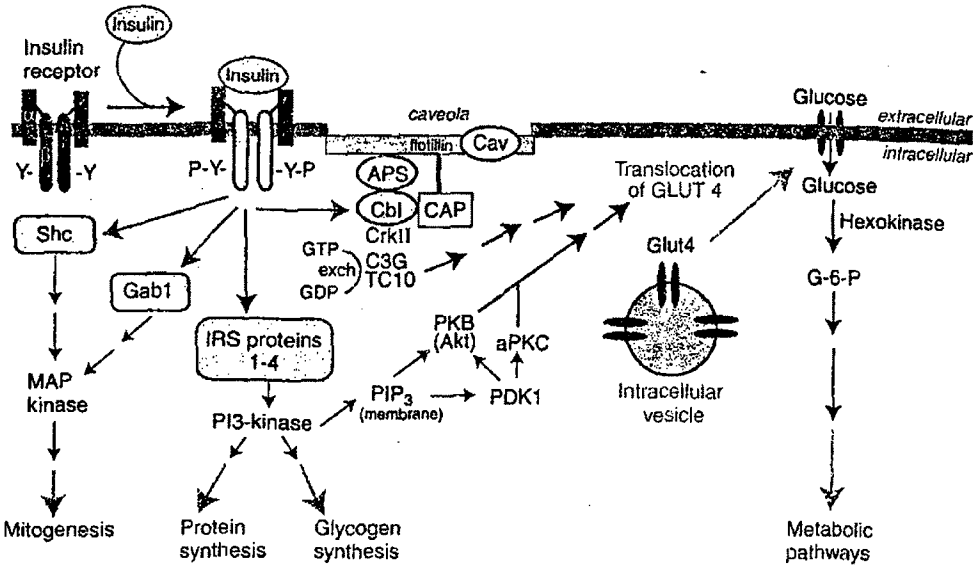

Figure 60–3. ***Pathways of insulin signaling.*** The binding of insulin to its plasma membrane receptor activates a cascade of downstream signaling events. Insulin binding activates the intrinsic tyrosine kinase activity of the receptor dimer, resulting in the tyrosine phosphorylation (Y-P) of the receptor's β subunits and a small number of specific substrates (light blue shapes): the Insulin Receptor Substrate (IRS) proteins, Gab-1 and SHC; within the membrane, a caveolar pool of insulin receptor phosphorylates caveolin (Cav), APS, and Cbl. These tyrosine-phosphorylated proteins interact with signaling cascades via SH2 and SH3 domains to mediate the effects of insulin, with specific effects of insulin resulting from each pathway. In target tissues such as skeletal muscle and adipocytes, a key event is the translocation of the Glut4 glucose transporter from intracellular vesicles to the plasma membrane; this translocation is stimulated by both the caveolar and non-caveolar pathways. In the non-caveolar pathway, the activation of PI3K is crucial, and PKB/Akt (anchored at the membrane by PIP3) and/or an atypical form of PKC is involved. In the caveolar pathway, the caveolar protein flotillin localizes the signaling complex to the caveola; the signaling pathway involves series of SH2 domain interactions that add the adaptor protein CrkII, the guanine nucleotide exchange protein C3G, and small GTP-binding protein, TC10. The pathways are inactivated by specific phosphoprotein phosphatases (eg, PTB1B) and possibly by actions of ser/thr protein kinases. In addition to the actions shown, insulin also stimulates the plasma membrane Na^+,K^+-ATPase by a mechanism that is still being elucidated; the result is an increase in pump activity and a net accumulation of K^+ in the cell. Abbreviations: APS, adaptor protein with PH and SH2 domains; CAP, Cbl associated protein; CrkII, chicken tumor virus regulator of kinase II; Glut4, glucose transporter 4; Gab-1, Grb-2 associated binder; MAP kinase, mitogen-activated protein kinase; PDK, phosphoinositide-dependent kinase; PI3 kinase, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol trisphosphate; PKB, protein kinase B (also called Akt); aPKC, atypical isoform of protein kinase C; Y, tyrosine residue; Y-P, phosphorylated tyrosine residue.

tion with GLUT4 in skeletal and cardiac muscle and in adipose tissue. Like GLUT4, hexokinase II is regulated transcriptionally by insulin.

G-6-P is a branch-point substrate that can enter several pathways. Thus, following isomerization to G-1-P, G-6-P can be stored as glycogen (insulin enhances the activity of glycogen synthase); G-6-P can enter the glycolytic pathway (leading to ATP production); and G-6-P can also enter the pentose phosphate pathway (providing NADPH for reductive syntheses, for the xenobiotic metabolizing activities of CYPs, and for maintenance of reduced glutathione). Effects of insulin on cellular metabolic enzymes are myriad and generally are mediated via the activities of protein kinases and phosphoprotein phosphatases that are enhanced following insulin treatment. Figure 60–3 shows the initial signaling events following the binding of insulin to its membrane receptor.

Regulation of Gene Transcription. A major action of insulin is the regulation of transcription of specific genes. More than a hundred genes are known to be regulated by insulin (O'Brien and Granner, 1996), although the mechanisms of regulation are still being worked out. As an example, insulin inhibits the transcription of phosphoenolpyruvate carboxykinase, contributing to insulin's inhibition of gluconeogenesis; this effect of insulin may explain why the liver overproduces glucose in the insulin-resistant state that is characteristic of type 2 DM.

The Insulin Receptor. Insulin initiates its actions by binding to a cell-surface receptor. Such receptors are present in virtually all mammalian cells, including not only the classic targets for insulin action (*i.e.*, liver, muscle, and fat) but also such nonclassic targets as circulating

blood cells, neurons, and gonadal cells. The number of receptors varies from as few as 40 per cell on erythrocytes to 300,000 per cell on adipocytes and hepatocytes.

The insulin receptor is a large transmembrane glycoprotein composed of two 135,000-dalton α subunits (719 or 731 amino acids, depending on whether a 12-amino-acid insertion has occurred through alternate RNA splicing) and two 95,000-dalton β subunits (620 amino acids); the subunits are linked by disulfide bonds to form a β-α-α-β heterotetramer (Figure 60–3) (Virkamäki *et al.*, 1999). Both subunits are derived from a single-chain precursor molecule that contains the entire sequence of the α and β subunits separated by a processing site consisting of four basic amino acid residues. The α subunits are entirely extracellular and contain the insulin-binding domain (*see* above), whereas the β subunits are transmembrane proteins that possess tyrosine protein kinase activity. After insulin is bound, receptors aggregate and are internalized rapidly. Since bivalent (but not monovalent) anti-insulin receptor antibodies cross-link adjacent receptors and mimic the rapid actions of insulin, it has been suggested that receptor dimerization is essential for signal transduction. After internalization, the receptor may be degraded or recycled back to the cell surface.

Tyrosine Phosphorylation and the Insulin Action Cascade. Receptors for insulin and IGF-1 belong to the family of receptor tyrosine kinases, in common with many growth factor receptors.

The activated receptors undergo autophosphorylation, which seems to activate their tyrosine kinase activity toward other substrates, principally the four insulin receptor substrates IRS-1 through 4 and Shc (White, 2002). The tyrosine phosphorylated IRS proteins direct the recruitment of signaling cascades *via* the interaction of SH2 domains with phosphotyrosines, recruiting such proteins as SHP2, Grb2, and SOS and resulting in the activation of MAP kinases and PI_3-kinase, which transduce many of insulin's cellular effects.

Insulin signaling is complicated by the fact that the IGF-1 receptor resembles the insulin receptor and uses similar signaling pathways; furthermore, the two receptors bind each other's ligand, albeit with lower affinity. In addition, IGF-1 and insulin-receptor heterodimers can combine to form hybrid heterotetramers.

The tyrosine kinase activity of the insulin receptor is required for signal transduction. Mutation of the insulin receptor with modification of the ATP-binding site or replacement of the tyrosine residues at major sites of autophosphorylation decreases both insulin-stimulated kinase activity and the cellular response to insulin. An insulin receptor incapable of autophosphorylation is biologically inert. A polymorphism in the human IRS-1, G972R, is associated with insulin resistance and increased risk of type 2 DM; this polymorphic IRS-1 appears to act as an inhibitor of the insulin-receptor tyrosine kinase (McGettrick *et al.*, 2005). In intact cells, the insulin receptor also is phosphorylated on serine and threonine residues, presumably by protein kinase C (PKC) and protein kinase A (PKA). Such phosphorylation inhibits the tyrosine kinase activity of the insulin receptor.

Diabetes Mellitus and the Physiological Effects of Insulin

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease. Most patients can be classified clinically as having either type 1 or type 2 DM (Expert Committee on the Diagnosis and Treatment of Diabetes Mellitus, 2003). DM or carbohydrate intolerance also is associated with certain other conditions or syndromes (Table 60–1). Criteria for the diagnosis of DM have been proposed by several medical organizations. The American Diabetes Association (ADA) criteria include symptoms of DM (*e.g.*, polyuria, polydipsia, and unexplained weight loss) and a random plasma glucose concentration of greater than 200 mg/dl (11.1 mM), a fasting plasma glucose concentration of greater than 126 ml/dl (7 mM), or a plasma glucose concentration of greater than 200 mg/dl (11 mM) 2 hours after the ingestion of an oral glucose load (Expert Committee on the Diagnosis and Treatment of Diabetes Mellitus, 2003).

The incidence of each type of diabetes varies widely throughout the world. In the United States, about 5% to 10% of all diabetic patients have type 1 DM, with an incidence of 18 per 100,000 inhabitants per year. A similar incidence is found in the United Kingdom. The incidence of type 1 DM in Europe varies with latitude. The highest rates occur in northern Europe (Finland, 43 per 100,000) and the lowest in the south (France and Italy, 8 per 100,000). The one exception to this rule is the small island of Sardinia, close to Italy, which has an incidence of 30 per 100,000. However, even the relatively low incidence rates of type 1 DM in southern Europe are far higher than the rates in Japan (1 per 100,000 inhabitants).

The vast majority of diabetic patients have type 2 DM. In the United States, about 90% of all diabetic patients have type 2 DM. Incidence rates of type 2 DM increase with age, with a mean rate of about 440 per 100,000 per year by the sixth decade in males in the United States. Ethnicity within a country also can influence the incidence of type 2 DM; the mean rate in African-American males is 540 per 100,000, and that in Pima Indians is about 5000 per 100,000. Unlike those for type 1 DM, the incidence rates for type 2 DM are lower in northern Europe (100 to 250 per 100,000) than in the south (800 per 100,000). Although prevalence data exist for type 2 DM, these numbers almost certainly are underestimates because 33% of all cases are undiagnosed.

There are more than 125 million persons with diabetes in the world today, and by 2010, this number is expected to approach 220 million (Amos *et al.*, 1997). Both type 1 and type 2 DM are increasing in frequency. The reason for the increase of type 1 DM is not known. The genetic basis for type 2 DM cannot change in such a short time; thus other contributing factors, including increasing age, obesity, sedentary lifestyle, and low birth weight, must account for this dramatic increase. In addition, type 2 DM is being diagnosed with remarkable frequency in preadolescents and adolescents. Up to 45% of newly diagnosed children and adolescents have type 2 DM.

In certain tropical countries, the most common cause of diabetes is chronic pancreatitis associated with nutritional or toxic factors (a form of secondary diabetes). Very rarely, diabetes results from point mutations in the insulin gene (Chan *et al.*, 1987). Amino acid substi-

Table 60–1
Different Forms of Diabetes Mellitus

General—genetic and other factors not precisely defined
- *Type 1 diabetes mellitus* (formerly called insulin-dependent diabetes mellitus)
  - Autoimmune type 1 diabetes mellitus (type 1A)
  - Non-autoimmune or idiopathic type 1 diabetes mellitus (type 1B)
- *Type 2 diabetes mellitus* (formerly called non-insulin-dependent diabetes mellitus)

Specific—defined gene mutations
- Maturity-onset diabetes of youth (MODY)
  - MODY 1 hepatic nuclear factor 4α (*HNF4A*) gene mutations
  - MODY 2 glucokinase (*GCK*) gene mutations
  - MODY 3 hepatic nuclear factor 1α (*TCF1*) gene mutations
  - MODY 4 insulin promoter factor 1 (*IPF1*) gene mutations
  - MODY 5 hepatic nuclear factor 1β (*HNF1β*) gene mutations
  - MODY 6 neurogenic differentiation 1 (*NEUROD1*) gene mutation
  - MODY X unidentified gene mutation(s)
- Maternally inherited diabetes and deafness (MIDD)
  - Mitochondrial leucine tRNA gene mutations
- Insulin gene mutations
- Insulin receptor gene mutations

Diabetes secondary to pancreatic disease
- Chronic pancreatitis
- Surgery
- Tropical diabetes (chronic pancreatitis associated with nutritional and/or toxic factors)

Diabetes secondary to other endocrinopathies
- Cushing's disease
- Glucocorticoid administration
- Acromegaly

Diabetes secondary to immune suppression

Diabetes associated with genetic syndromes; *e.g.*, Prader-Willi syndrome

Diabetes associated with drug therapy (*see* Table 60–5)

tutions from such mutations may result in insulins with lower potency or may alter the processing of proinsulin to insulin (*see* above). Other single-gene mutations cause the several types of MODY (Hattersley, 1998) and maternally inherited diabetes and deafness (van den Ouwenland *et al.*, 1992) (Table 60–1).

There are genetic and environmental components that affect the risk of developing either type 1 or type 2 DM. A positive family history of type 2 DM is predictive for the disease. Studies of identical twins show 70% to 80% concordance for developing type 2 DM. Furthermore, there is a high prevalence of type 2 DM in offspring of parents with the disease (up to 70%) as well as in siblings of affected individuals. Persons more than 20% over their ideal body weight also have a greater risk of developing type 2 DM. In fact, 80% to 90% of type 2 DM subjects in the United States are obese. Certain ethnic groups have a higher incidence of type 2 DM (*e.g.*, American Indians, African-Americans, Hispanics, and Polynesian Islanders). In addition, previously identified impaired glucose tolerance, gestational diabetes, hypertension, or significant hyperlipidemia is associated with an increased risk of type 2 DM. These data suggest a strong genetic basis for type 2 DM, but the genetic mechanism(s) involved are not known. A pancreatic β-cell defect and a reduction in tissue sensitivity to insulin both are required before overt type 2 DM is apparent. However, type 2 DM is an extremely heterogeneous disease, and it is likely that a number of different genes are involved. In addition, environmental factors may play a role. Type 2 DM thus is a multifactorial disease. Any combination of genetic and environmental factors that exceeds a threshold can result in type 2 DM. The genetic basis for type 2 DM in a small subset of patients has been established. MODY2 is a rare disorder that is inherited as an autosomal dominant trait and is caused by mutations of the glucokinase gene. Because of decreased glucokinase activity, these patients have an increased glycemic threshold for insulin release that results in persistent mild hyperglycemia. This and other rare genetic forms of MODY are quite distinct from the usual type 2 DM (Table 60–1).

With type 1 DM, the concordance rate for identical twins is only 25% to 50%; this suggests that environmental as well as genetic influences have an important role in the disease. However, the known genetic factors in type 1 DM relate to the genes that control the immune response. There is considerable evidence that type 1 DM involves an autoimmune attack on the pancreatic β cell. Antibodies to islet cell antigens are detected in up to 80% of patients with type 1 DM shortly after diagnosis or even prior to the onset of clinical disease. The antibodies are directed at both cytoplasmic and membrane-bound antigens and include islet cell antibodies and antibodies directed against insulin (IAAs), glutamic acid decarboxylase-65 and -67 (GAD-65 and -67), heat-shock protein 65 (HSP-65), and tyrosine phosphatase–like protein (IA-2 or IA-2B).

Although these autoantibodies are correlated with the clinical expression of type 1 DM, it is controversial whether they can predict the development of clinical diabetes. Most prospective studies designed to determine if type 1 DM can be predicted on the basis of antibodies have been performed in healthy first-degree relatives of diabetic patients. These studies have determined that the presence of IAAs confers only a small risk for the development of type 1 DM. On the other hand, the presence of high-titer islet-cell antibodies (ICAs) and GAD antibodies or ICAs combined with IAAs confers a very high risk for the development of type 1 DM in first-degree relatives (Verge *et al.*, 1996).

Since most of the studies aimed at predicting the development of type 1 DM have been carried out in first-degree relatives of diabetic patients, it is not known whether the occurrence of ICAs in individuals from the general population confers a similar risk for development of clinical diabetes. Most available data indicate that the presence of ICAs in individuals from the general population

associated with a lower risk of developing type 1 DM. However, as in first-degree relatives of type 1 DM patients, it may be that the presence of more than one form of autoantibody in individuals from the general population is a more powerful predictor of the development of clinical diabetes. Individuals with type 1 DM also tend to have antibodies directed toward other endocrine tissues, including the adrenal, parathyroid, and thyroid glands, and have an increased incidence of other autoimmune diseases.

Type 1 DM is associated with specific human leukocyte antigen (HLA) alleles, especially at the B and DR loci (Florez *et al.*, 2003). Approximately 90% of patients with type 1 DM are positive for HLA-DR3 or -DR4, as compared with only 40% of the general population. Compound heterozygotes appear to be at particular risk. In contrast, the haplotype HLA-DR2 appears to be negatively associated with the occurrence of the disease. A polymorphism of the HLA-DQβ chain at position 57 correlates even more closely with susceptibility to diabetes (Todd *et al.*, 1987). Type 1 DM is associated with alleles coding for alanine, valine, or serine at position 57 in the HLA-DQβ chain, whereas aspartic acid in this position is negatively correlated with the disease in Caucasians. These findings implicate both humoral and cell-mediated immune mechanisms in the etiology of type 1 DM.

The trigger for the immune response remains unknown. The identification of triggering agents is difficult because autoimmune destruction of pancreatic β cells may occur over a period of many months or several years before the onset of overt disease. In about 10% of new cases of type 1 DM, there is no evidence of autoimmune insulitis (Imagawa *et al.*, 2000). The ADA and the World Health Organization (WHO) therefore subdivide this disease into autoimmune (1A) and idiopathic (1B) subtypes. Whatever the causes, the final result in type 1 DM is an extensive and selective loss of pancreatic β cells and a state of absolute insulin deficiency.

The situation in type 2 DM is not so clear-cut. Most studies indicate that there is reduced β-cell mass in type 2 DM patients. Obesity, duration of diabetes, and prevailing hyperglycemia potentially can confound interpretation of data, but studies that have controlled for these variables have reported an approximately 50% reduction in β-cell volume in type 2 DM patients compared with nondiabetic control subjects. Owing to the heterogeneous nature of type 2 DM, mean 24-hour plasma concentrations of insulin in patients have been reported to vary from low to even normal relative to values in control subjects. Of note, standard insulin radioimmunoassays detect proinsulin and processing intermediates. Studies in which specific insulin and proinsulin assays have been used (Temple *et al.*, 1989) have revealed that "true" insulin values in "hyperinsulinemic" type 2 DM patients are either no greater or distinctly less than values in control subjects. Therefore, increased amounts of proinsulin have confounded the appreciation of subnormal insulin levels in type 2 DM patients. Furthermore, even apparently "normal" values of plasma insulin in a hyperglycemic type 2 DM patient are considerably reduced relative to the insulin levels that would be observed in a similarly hyperglycemic nondiabetic individual.

In healthy persons, the contribution of proinsulin to basal immunoreactive insulin levels is low. Proinsulin intermediates make up about 10% of the total immunoreactive insulin in the portal vein. However, owing to its long half-life (about 44 minutes) and tenfold slower metabolic clearance, proinsulin and intermediates make up about 20% of circulating immunoreactive insulin. This amount is physiologically trivial because proinsulin has only about 5% of the metabolic effect of insulin (Davis *et al.*, 1991). Nevertheless, plasma proinsulinlike molecules are increased in type 2 DM to about 20% or more of total immunoreactive insulin. Furthermore, proinsulin levels increase in response to any β-cell stimulation.

Type 2 DM also is associated with several distinct defects in insulin secretion. At diagnosis, virtually all persons with type 2 DM have a profound defect in first-phase insulin secretion in response to an intravenous glucose challenge. The responses to other secretagogues (*e.g.*, isoproterenol or arginine) are preserved, although there is less potentiation by glucose. Some of these β-cell abnormalities in type 2 DM may be secondary to desensitization by chronic hyperglycemia. The relationship between fasting glycemia and insulinemia in type 2 DM subjects is complex. Patients who have fasting blood glucose levels of 6 to 10 mM (108 to 180 mg/dl) have fasting and stimulated insulin values equal to those of euglycemic control subjects. More severely hyperglycemic subjects are frankly hypoinsulinemic.

Virtually all forms of DM are caused by a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance). These abnormalities lead to alterations in the metabolism of carbohydrates, lipids, ketones, and amino acids; the central feature of the syndrome is hyperglycemia (Figure 60–4).

Insulin lowers the concentration of glucose in blood by inhibiting hepatic glucose production and by stimu-

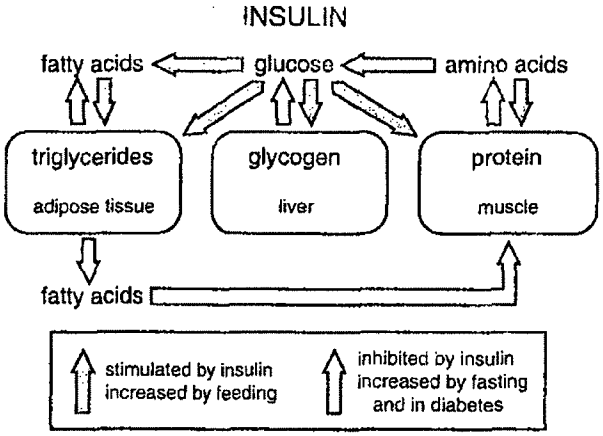

Figure 60–4. *Overview of insulin action.* Insulin stimulates glucose storage in the liver as glycogen and in adipose tissue as triglycerides and amino acid storage in muscle as protein; it also promotes utilization of glucose in muscle for energy. These pathways, which also are enhanced by feeding, are indicated by the solid blue arrows. Insulin inhibits the breakdown of triglycerides, glycogen, and protein and the conversion of amino acids to glucose (gluconeogenesis), as indicated by the white arrows. These pathways are increased during fasting and in diabetic states. The conversion of amino acids to glucose and of glucose to fatty acids occurs primarily in the liver.

Table 60–2
Hypoglycemic Actions of Insulin

LIVER	MUSCLE	ADIPOSE TISSUE
Inhibits hepatic glucose production (decreases gluconeogenesis and glycogenolysis)	Stimulates glucose uptake	Stimulates glucose uptake (amount is small compared to muscle)
Stimulates hepatic glucose uptake	Inhibits flow of gluconeogenic precursors to the liver (*e.g.*, alanine, lactate, and pyruvate)	Inhibits flow of gluconeogenic precursor to liver (glycerol) and reduces energy substrate for hepatic gluconeogenesis (nonesterfied fatty acids)

lating the uptake and metabolism of glucose by muscle and adipose tissue (Table 60–2). These two important effects occur at different concentrations of insulin. Production of glucose is inhibited half maximally by an insulin concentration of about 20 μunits/ml, whereas glucose utilization is stimulated half maximally at about 50 μunits/ml.

In both types of diabetes, glucagon (the levels of which are elevated in untreated patients) opposes the hepatic effects of insulin by stimulating glycogenolysis and gluconeogenesis, but it has relatively little effect on peripheral utilization of glucose. Thus, in the diabetic patient with insulin deficiency or insulin resistance and hyperglucagonemia, there is an increase in hepatic glucose production, a decrease in peripheral glucose uptake, and a decrease in the conversion of glucose to glycogen in the liver (DeFronzo *et al.*, 1992).

Alterations in secretion of insulin and glucagon also profoundly affect lipid, ketone, and protein metabolism. At concentrations below those required to stimulate glucose uptake, insulin inhibits the hormone-sensitive lipase in adipose tissue and thus inhibits the hydrolysis of triglyceride stores. This counteracts the lipolytic action of catecholamines, cortisol, and growth hormone and reduces the concentrations of glycerol (a substrate for gluconeogenesis) and free fatty acids (a substrate for production of ketone bodies and a necessary fuel for gluconeogenesis). These actions of insulin are deficient in the diabetic patient, leading to increased gluconeogenesis and ketogenesis.

The liver produces ketone bodies by oxidation of free fatty acids to acetyl CoA, which then is converted to acetoacetate and β-hydroxybutyrate. The initial step in fatty acid oxidation is transport of the fatty acid into the mitochondria. This involves the interconversion of the coenzyme A (CoA) and carnitine esters of fatty acids by the enzyme acylcarnitine transferase. The activity of this enzyme is inhibited by intramitochondrial malonyl CoA, one of the products of fatty acid synthesis. Under normal conditions, insulin inhibits lipolysis, stimulates fatty acid synthesis (thereby increasing the concentration of malonyl CoA), and decreases the hepatic concentration of carnitine; these factors all decrease the production of ketone bodies. Conversely, glucagon stimulates ketone body production by increasing fatty acid oxidation and decreasing concentrations of malonyl CoA. In the diabetic patient, particularly the patient with type I DM, the consequences of insulin deficiency and glucagon excess provide a hormonal milieu that favors ketogenesis and, in the absence of appropriate treatment, may lead to ketonemia and acidosis.

Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. This enzyme hydrolyzes triglycerides present in very low density lipoproteins (VLDL) and chylomicrons, resulting in release of intermediate-density lipoprotein (IDL) particles (*see* Chapter 35). The IDL particles are converted by the liver to the more cholesterol-rich low-density lipoproteins (LDL). Thus, in the untreated or undertreated diabetic patient, hypertriglyceridemia and hypercholesterolemia often occur. In addition, deficiency of insulin may be associated with increased production of VLDL.

The important role of insulin in protein metabolism usually is evident clinically only in diabetic patients with persistently poor control of their disease. Insulin stimulates amino acid uptake and protein synthesis and inhibits protein degradation in muscle and other tissues; it thus causes a decrease in the circulating concentrations of most amino acids. Glutamine and alanine are the major amino

acid precursors for gluconeogenesis. Insulin lowers alanine concentrations during hyperinsulinemic euglycemic conditions. The rate of appearance of alanine is maintained in part by the enhanced rate of transamination of pyruvate to alanine. However, alanine utilization greatly exceeds production (owing to increased hepatic uptake and fractional extraction of the amino acid), and this results in a fall of peripheral alanine levels. In poorly controlled diabetics, there is increased conversion of alanine to glucose, contributing to the enhanced rate of gluconeogenesis. The increased conversion of amino acids to glucose also results in increased production and excretion of urea and ammonia. In addition, there are increased circulating concentrations of branched-chain amino acids as a result of increased proteolysis, decreased protein synthesis, and increased release of branched-chain amino acids from the liver.

A nearly pathognomonic feature of diabetes mellitus is thickening of the capillary basement membrane and other vascular changes that occur during the course of the disease. The cumulative effect is progressive narrowing of the vessel lumina, causing inadequate perfusion of critical regions of certain organs. The matrix is expanded in many vessel walls, in the basement membrane of the retina, and in the mesangial cells of the renal glomerulus (McMillan, 1997). Cellular proliferation in many large vessels further contributes to luminal narrowing. These pathological changes contribute to some of the major complications of diabetes, including premature atherosclerosis, intercapillary glomerulosclerosis, retinopathy, neuropathy, and ulceration and gangrene of the extremities.

It is hypothesized that the factor responsible for the development of most complications of diabetes is the prolonged exposure of tissues to elevated concentrations of glucose. Prolonged hyperglycemia results in the formation of advanced glycation end products (Beisswenger *et al.*, 1995). These macromolecules are thought to induce many of the vascular abnormalities that result in the complications of diabetes (Brownlee, 1995). The results from the Diabetes Control and Complications Trial (DCCT) definitively answered this question: Most diabetic complications arise from prolonged exposure of tissue to elevated glucose concentrations.

The DCCT (DCCT Research Group, 1993) was a multicenter, randomized clinical trial designed to compare intensive and conventional diabetes therapies with regard to their effects on the development and progression of the early vascular and neurologic complications of type 1 DM. The intensive therapy regimen was designed to achieve blood glucose values as close to the normal range as possible with three or more daily insulin injections or with an external insulin pump. Conventional therapy consisted of one or two insulin injections daily. Two groups of patients were studied to answer separate but related questions. The first question was whether or not intensive therapy could prevent the development of diabetic complications such as retinopathy, nephropathy, and neuropathy (primary prevention). The second was whether or not intensive therapy could slow the progression of existing complications of diabetes (secondary intervention).

In the primary prevention group, intensive therapy reduced the mean risk for the development of retinopathy by 76% compared with conventional therapy. In the secondary intervention group, intensive therapy slowed the progression of retinopathy by 54%. Intensive therapy reduced the risk of nephropathy by 34% in the primary prevention group and by 43% in the secondary intervention group. Similarly, neuropathy was reduced by about 60% in both the primary prevention and secondary intervention groups. Intensive therapy reduced the development of hypercholesterolemia by 34% overall. Because of the relative youth of the patients, it was predicted that the detection of treatment-related differences in rates of macrovascular events would be unlikely. However, intensive therapy reduced the risk of macrovascular disease by 41% in the combined groups. These results established unequivocally that improving day-to-day glycemic control in type 1 DM patients can reduce and slow the development of diabetic complications dramatically. A follow-up study showed that the reduction in the risk of progressive retinopathy and nephropathy persists for at least 4 years, even if tight glycemic control was not maintained (DCCT Research Group, 2000).

A serious complication of intensive therapy was an increased incidence of severe hypoglycemia. Patients receiving intensive therapy had a threefold greater incidence of severe hypoglycemia (blood glucose concentration below 50 mg/dl or 2.8 mM and needing external resuscitative assistance) and hypoglycemic coma than did conventionally treated subjects. Therefore, the present guidelines for treatment given by the ADA include a contraindication for implementing tight metabolic control in infants younger than 2 years old and an extreme caution in children between 2 and 7 years of age because hypoglycemia may impair brain development. Older patients with significant arteriosclerosis also may be vulnerable to permanent injury from hypoglycemia.

The DCCT was performed in relatively young type 1 DM patients. The question was asked whether intensive therapy would provide similar benefits to typical middle-aged or elderly patients with type 2 DM. The results of the DCCT indeed also apply to patients with type 2 DM (U.K. Prospective Diabetes Study Group, 1998a, 1998b). The eye, kidney, and nerve abnormalities appear similar in type 1 and type 2 DM, and it is likely that the same or similar underlying mechanisms of disease apply. However, because of a higher prevalence of macrovascular disease, older patients with type 2 DM may be more vulnerable to serious consequences of hypoglycemia. Thus, as is the case for everyone with diabetes, treatment of type 2 DM patients must be tailored to the individual. Nevertheless, the results of the DCCT and U.K. Prospective Diabetes Study (UKPDS) suggest that many otherwise healthy patients with type 2 DM should attempt to achieve tight metabolic control.

The toxic effects of hyperglycemia may be the result of accumulation of non-enzymatically glycosylated products and osmotically active sugar alcohols such as sorbitol in tissues; the effects of glucose on cellular metabolism also may be responsible (Brownlee, 1995). The covalent reac-

tion of glucose with hemoglobin provides a convenient method to determine an integrated index of the glycemic state. Hemoglobin undergoes glycosylation on its amino-terminal valine residue to form the glucosyl valine adduct of hemoglobin, termed *hemoglobin* A_{1c} (Brownlee, 1995). The half-life of the modified hemoglobin is equal to that of the erythrocyte (about 120 days). Since the amount of glycosylated protein formed is proportional to the glucose concentration and the time of exposure of the protein to glucose, the concentration of hemoglobin A_{1c} in the circulation reflects the severity of the glycemic state over an extended period (4 to 12 weeks) prior to sampling. Thus a rise in hemoglobin A_{1c} from 5% to 10% suggests a prolonged doubling of the mean blood glucose concentration. Although this assay is used widely, measurement of the glycosylation of proteins with somewhat shorter survival times (*e.g.*, albumin) also has proven useful in the management of pregnant diabetic patients.

Glycosylated products accumulate in tissues and eventually may form cross-linked proteins termed *advanced glycosylation end products* (Beisswenger *et al.*, 1995). Such nonenzymatic glycosylation may be directly responsible for expansion of the vascular matrix and the vascular complications of diabetes. This process also may explain the modified cellular proliferative activity in vascular lesions of diabetic patients because macrophages appear to have receptors for advanced glycosylation end products. Binding of such proteins to macrophages in these lesions may stimulate the production of cytokines such as tumor necrosis factor α and interleukin 1 (IL-1), which, in turn, induce degradative and proliferative cascades in mesenchymal and endothelial cells, respectively.

Other explanations for the toxic manifestations of hyperglycemia may exist. Intracellular glucose is reduced to its corresponding sugar alcohol, sorbitol, by the enzyme aldose reductase, and the rate of production of sorbitol is determined by the ambient glucose concentration. This is particularly true in tissues such as the lens, retina, arterial wall, and Schwann cells of peripheral nerves. In diabetic human beings and rodents, these tissues have increased intracellular concentrations of sorbitol, which may contribute to an increased osmotic effect and tissue damage. Inhibitors of aldose reductase currently are being evaluated for treatment of diabetic neuropathy and retinopathy. The results of studies with these agents thus far have been somewhat conflicting and inconclusive (reviewed by Frank, 1994).

In neural tissue and perhaps in other tissues, glucose competes with myoinositol for transport into cells. Reduction of cellular concentrations of myoinositol may contribute to altered nerve function and neuropathy. Hyperglycemia also may enhance the *de novo* synthesis of diacylglycerol, which could facilitate persistent activation of protein kinase C.

Insulin Therapy

Insulin is the mainstay for treatment of virtually all type 1 DM and many type 2 DM patients. When necessary, insulin may be administered intravenously or intramuscularly; however, long-term treatment relies predominantly on subcutaneous injection of the hormone. Subcutaneous administration of insulin differs from physiological secretion of insulin in at least two major ways: The kinetics do not reproduce the normal rapid rise and decline of insulin secretion in response to ingestion of nutrients, and the insulin diffuses into the peripheral circulation instead of being released into the portal circulation; the direct effect of secreted insulin on hepatic metabolic processes thus is eliminated. Nonetheless, when such treatment is performed carefully, considerable success is achieved.

Preparations of insulin can be classified according to their duration of action into short, intermediate, and long acting and by their species of origin—human or porcine. Human insulin (HUMULIN, NOVOLIN) is now widely available as a result of its recombinant production. Porcine insulin differs from human insulin by one amino acid (alanine instead of threonine at the carboxy terminal of the B chain, *i.e.*, in position B30. Prior to the mid-1970s, commercially available insulin preparations contained proinsulin or glucagonlike substances, pancreatic polypeptide, somatostatin, and vasoactive intestinal peptides. These contaminants were avoided with the advent of monocomponent porcine insulins. During the late 1970s, intense work was carried out on the development of biosynthetic human insulin. During the last decade, human insulin rapidly has become the standard form of therapy, and beef insulin products have been discontinued in the United States.

The physicochemical properties of human and porcine insulins differ owing to their different amino acid sequences. Human insulin, produced using recombinant DNA technology, is more soluble than porcine insulin in aqueous solution owing to the presence of threonine (instead of alanine), with its extra hydroxyl group. The vast majority of preparations now are supplied at neutral pH, which improves stability and permits storage for several days at a time at room temperature.

Table 60-
Properties

TYPE

Rapid
Regulau
(crys
Lispro
Aspart
Glulisin
Intermed
NPH (is
Lente
Slow
Ultralen
Protamii
Glargine

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Classification of
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nephrine, norepi
plasma glucose
normal counter-r
autonomic neurc

Table 60–3
Properties of Currently Available Insulin Preparations

TYPE	APPEARANCE	ADDED PROTEIN	ZINC CONTENT, mg/100 units	BUFFER*	Action, Hours†		
					ONSET	PEAK	DURATION
Rapid							
Regular soluble (crystalline)	Clear	None	0.01–0.04	None	0.5–0.7	1.5–4	5–8
Lispro	Clear	None	0.02	Phosphate	0.25	0.5–1.5	2–5
Aspart	Clear	None	0.0196	Phosphate	0.25	0.6–0.8	3–5
Glulisine	Clear	None	None	None	—	0.5–1.5	1–2.5
Intermediate							
NPH (isophane)	Cloudy	Protamine	0.016–0.04	Phosphate	1–2	6–12	18–24
Lente	Cloudy	None	0.2–0.25	Acetate	1–2	6–12	18–24
Slow							
Ultralente	Cloudy	None	0.2–0.25	Acetate	4–6	16–18	20–36
Protamine zinc	Cloudy	Protamine	0.2–0.25	Phosphate	4–6	14–20	24–36
Glargine	Clear	None	0.03	None	2–5	5–24	18–24

*Most insulin preparations are supplied at pH 7.2–7.4. Glargine is supplied at a pH of 4.0. †These are approximate figures. There is considerable variation from patient to patient and from time to time in a given patient.

Unitage. For therapeutic purposes, doses and concentrations of insulin are expressed in units. This tradition dates to the time when preparations of the hormone were impure, and it was necessary to standardize them by bioassay. One unit of insulin is equal to the amount required to reduce the concentration of blood glucose in a fasting rabbit to 45 mg/dl (2.5 mM). The current international standard is a mixture of bovine and porcine insulins and contains 24 units/mg. Homogeneous preparations of human insulin contain between 25 and 30 units/mg. Almost all commercial preparations of insulin are supplied in solution or suspension at a concentration of 100 units/ml, which is about 3.6 mg insulin per milliliter (0.6 mM). Insulin also is available in a more concentrated solution (500 units/ml) for patients who are resistant to the hormone.

Classification of Insulins. *Short-* and *rapid-acting insulins* are solutions of *regular, crystalline zinc insulin (insulin injection)* dissolved usually in a buffer at neutral pH. These preparations have the most rapid onset of action but the shortest duration (Table 60–3). Short-acting insulin (*i.e.*, regular or soluble) usually should be injected 30 to 45 minutes before meals. Regular insulin also may be given intravenously or intramuscularly. After intravenous injection, there is a rapid fall in the blood glucose concentration, which usually reaches a nadir in 20 to 30 minutes. In the absence of a sustained infusion of insulin, the hormone is cleared rapidly, and counter-regulatory hormones (*e.g.*, glucagon, epinephrine, norepinephrine, cortisol, and growth hormone) restore plasma glucose to baseline in 2 to 3 hours. In the absence of a normal counter-regulatory response (*e.g.*, in diabetic patients with autonomic neuropathy), plasma glucose will remain suppressed for many hours following an insulin bolus of 0.15 units/kg because the cellular actions of insulin are prolonged far beyond its clearance from plasma. Intravenous infusions of insulin are useful in patients with ketoacidosis or when requirements for insulin may change rapidly, such as during the perioperative period, during labor and delivery, and in intensive care situations (*see* below).

When metabolic conditions are stable, regular insulin usually is given subcutaneously in combination with an intermediate- or long-acting preparation. Short-acting insulin is the only form of the hormone that can be used in subcutaneous infusion pumps. Special buffered formulations of regular insulin have been made for the latter purpose that are less likely to crystallize in the tubing during the slow infusion associated with this type of therapy.

The native insulin monomers are associated as hexamers in currently available insulin preparations. These hexamers slow the absorption and reduce postprandial peaks of subcutaneously injected insulin. These pharmacokinetics stimulated the development of short-acting insulin analogs that retain a monomeric or dimeric configuration. A number of compounds have been investigated, and two, insulin *lispro* (HUMALOG) and insulin *aspart* (NOVOLOG), are available for clinical use (Hirsch, 2005). These analogs are absorbed three times more rapidly from subcutaneous sites than is human insulin. Consequently, there is a more rapid increase in plasma insulin concentrations and an earlier hypoglycemic response. Injection of the analogs 15 minutes before a meal affords glycemic control similar to that from an injection of human insulin given 30 minutes before the meal. The first commercially available short-acting analog was human insulin lispro. This analog is identical to human insulin except at positions B28 and B29, where the sequence of the two residues has been

reversed to match the sequence in IGF-1, which does not self-associate. Like regular insulin, lispro exists as a hexamer in commercially available formulations. Unlike regular insulin, lispro dissociates into monomers almost instantaneously following injection. This property results in the characteristic rapid absorption and shorter duration of action compared with regular insulin. Two therapeutic advantages have emerged with lispro as compared with regular insulin. First, the prevalence of hypoglycemia is reduced by 20% to 30% with lispro; second, glucose control, as assessed by hemoglobin A_{1c}, is modestly but significantly improved (0.3% to 0.5%) with lispro as compared with regular insulin.

Insulin aspart is formed by the replacement of proline at B28 with aspartic acid. This reduces self-association to that observed with lispro. Like lispro, insulin aspart dissociates rapidly into monomers following injection. Comparison of a single subcutaneous dose of aspart and lispro in a group of type 1 DM patients revealed similar plasma insulin profiles. In clinical trials, insulin aspart and insulin lispro have had similar effects on glucose control and hypoglycemia frequency, with lower rates of nocturnal hypoglycemia as compared with regular insulin (reviewed by Hirsh, 2005).

A third rapid-acting insulin analog called insulin *glulysine* has been approved for clinical use in the United States. In this compound, glutamic acid replaces lysine at B29, and lysine replaces asparagine at B23. Similar to the other two available rapid-acting analogs, this causes a reduction in self-association and rapid dissociation into active monomers. The time–action profile of insulin glulysine is similar to that of insulin aspart and lispro. Similar to insulin aspart, glulysine has been approved by the Food and Drug Administration (FDA) for continuous subcutaneous insulin infusion (CSII) pump use. Owing to their rapid onset, the fast-acting insulin analogs all may be injected immediately before or after a meal, which may confer considerable clinical advantages. Many individuals with diabetes consume smaller amounts of food than originally planned. This, in the presence of a previously injected dose of insulin that was based on a larger meal, could result in postprandial hypoglycemia. Thus, in patients who have gastroparesis or loss of appetite, injection of a rapid-acting analog postprandially, based on the amount of food actually consumed, may provide smoother glycemic control.

Clinical trials of inhaled insulin are underway in a number of countries. Early results demonstrate that inhaled insulin and short-acting subcutaneously-injected insulin provide similar postprandial glycemic control in patients with type 1 and type 2 DM. Patient satisfaction is uniformly high with inhaled insulin, and the prevalence of hypoglycemia is no higher (and may even be reduced) compared with regular (soluble) insulin. Smoking increases and asthma decreases absorption of inhaled insulin. Long-term safety data are awaited before registration approval of the compound will be given.

Intermediate-acting insulins are formulated to dissolve more gradually when administered subcutaneously; thus their durations of action are longer. The two preparations used most frequently are *neutral protamine Hagedorn (NPH) insulin (isophane insulin suspension)* and *lente insulin (insulin zinc suspension)*. NPH insulin is a suspension of insulin in a complex with zinc and protamine in a phosphate buffer. Lente insulin is a mixture of crystallized (ultralente) and amorphous (semilente) insulins in an acetate buffer, which minimizes the solubility of insulin. The pharmacokinetic properties of human intermediate-acting insulins are slightly different from those of porcine preparations. Human insulins have a more rapid onset and shorter duration of action than do porcine insulins. This difference may be related to the more hydrophobic nature of human insulin, or human and porcine insulins may interact differently with protamine and zinc crystals. This difference may create a problem with optimal timing for evening therapy; human insulin preparations taken before dinner may not have a duration of action sufficient to prevent hyperglycemia by morning. It should be noted that there is no evidence that lente or NPH insulin has different pharmacodynamic effects when used in combination with regular (soluble) insulin in a twice-a-day dosage regimen. Intermediate-acting insulins usually are given either once a day before breakfast or twice a day. In patients with type 2 DM, intermediate-acting insulin given at bedtime may help normalize fasting blood glucose. When lente insulin is mixed with regular insulin, some of the regular insulin may form a complex with the protamine or Zn^{2+} after several hours, and this may slow the absorption of the fast-acting insulin. NPH insulin does not retard the action of regular insulin when the two are mixed vigorously by the patient or when they are available commercially as a mixture (Davis *et al.*, 1991) (*see* below).

Ultralente insulin (extended insulin zinc suspension) and protamine zinc insulin suspension are long-acting insulins; they have a slower onset and a prolonged peak of action. These insulins have been advocated to provide a low basal concentration of insulin throughout the day. The long half-life of ultralente insulin makes it difficult to determine the optimal dosage because several days of treatment are required before a steady-state concentration of circulating insulin is achieved. Doses given once or twice daily are adjusted according to the fasting blood glucose concentration. Protamine zinc insulin is used rarely today because of its very unpredictable and prolonged course of action, and it is no longer available in the United States. Preparations of insulin that are available for clinical use in the United States are shown in Table 60–4.

In the vast majority of patients, insulin-replacement therapy includes intermediate- or long-acting insulin. A search for the ideal intermediate-acting insulin identified *human proinsulin* (HPI) as a promising candidate. Animal studies using *porcine proinsulin* indicated that the compound was a soluble intermediate-acting insulin agonist that had a greater suppressive effect on hepatic glucose production than on stimulation of peripheral glucose disposal. This profile of action appeared favorable for clinical use in diabetic subjects because unrestrained hepatic glucose production is a hallmark of the disease, and a liver-specific insulin would tend to reduce peripheral hyperinsulinemia and the attendant risk of hypoglycemia. Early studies with HPI in human beings confirmed its relatively specific action on hepatocytes and demonstrated that its duration of action was similar to that of NPH insulin. Preliminary results from clinical trials, however, indicated that HPI conferred no additional benefit over currently available human insulins, and all clinical studies soon were suspended because of a high incidence of myocardial infarction in HPI-treated subjects.

The pharmacokinetic limitations of ultralente insulin have prompted efforts to develop an insulin analog that does not have a significant peak in its action. Considerable research has been directed to the development of such a product. Insulin *glargine* (LANTUS) is the first long-acting analog of human insulin to be approved for clinical use in the United States and Europe. Insulin glargine is produced following two alterations of human insulin (Hirsch, 2005). Two arginine residues are added to the C terminus of the B chain, and an asparagine molecule in position A21 on the A chain is

Chapte

Table
Insulin

TYPE

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75%
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70%
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Table 60–4
Insulin Preparations Available in the United States

TYPE	HUMAN	PORCINE
Rapid		
Insulin injection (regular)	R, C	P, S
Lispro	R	—
Aspart	R	—
Glulisine	R	—
Intermediate		
Isophane insulin suspension (NPH)	R	P
Insulin zinc suspension (lente)	R	P
Slow		
Extended insulin zinc suspension (ultralente)	R	—
Insulin glargine	R	—
Mixtures		
70% NPH/30% Regular	R	—
50% NPH/50% Regular	R	—
75% Lispro Protamine/ 25% Lispro	R	—
70% Aspart Protamine/ 30% Aspart	R	—

ABBREVIATIONS: S, standard insulins; P, purified insulins; C, concentrated insulin; R, recombinant or semisynthetic human insulins.

replaced with glycine. Glargine is a clear solution with a pH of 4.0. This pH stabilizes the insulin hexamer and results in a prolonged and predictable absorption from subcutaneous tissues. Owing to insulin glargine's acidic pH, it cannot be mixed with currently available short-acting insulin preparations (*i.e.*, regular insulin, aspart, or lispro) that are formulated at a neutral pH. In clinical studies, insulin glargine results in less hypoglycemia, has a sustained "peakless" absorption profile, and provides a better once-daily 24-hour insulin coverage than ultralente or NPH insulin. Glargine may be administered at any time during the day with equivalent efficacy and no difference in the frequency of hypoglycemic episodes. Glargine does not accumulate after several injections. A recent study has demonstrated that duration of action remains at approximately 24 hours and intersubject variability actually improves after seven as compared to one injection.

Insulin glargine can be combined with various oral antihyperglycemic agents (*see* below) to effectively lower plasma glucose levels. Combination of glargine with *sulfonylureas* and/or *metformin* can reduce both fasting (basal) and postprandial glucose levels. It should be noted that the use of a long-acting basal insulin alone will not control postprandial glucose elevations in insulin-deficient type 1 or type 2 DM. Glargine has been shown in clinical studies to normalize fasting (postabsorptive) glucose levels following once-daily administration in patients with type 2 DM. Rarely, splitting the dose of glargine may be needed in very lean, insulin-sensitive type 1 DM patients in order to achieve good fasting (basal) glucose levels. Unlike traditional insulin preparations that are absorbed more rapidly from the abdomen than from the arm or leg, the site of administration does not influence the time–action profile of glargine. Similarly, exercise does not influence glargine's unique absorption kinetics, even when the insulin is injected into a working limb. Glargine binds with a slightly greater affinity to IGF-1 receptors as compared with human insulin. However, this slightly increased binding is still approximately two log scales lower than that of IGF-1. The deterioration of retinopathy in a few patients with type 2 DM in early clinical studies with glargine led to a concern that it might be involved in the development of retinopathy. However, none of the patients had optic disc swelling, which is pathognomonic of IGF-1 effects, suggesting that the finding was probably due to the well-recognized "glucose reentry phenomenon" that occurs with improvement of glycemic control rather than the insulin *per se*.

Other approaches to prolong the action of soluble insulin analogs are under investigation. One approach is the addition of a saturated fatty acid to the ε amino group of LysB29, yielding a myristoylated insulin called *insulin detemir* (Hirsch, 2005).

When insulin detemir is injected subcutaneously, it binds to albumin *via* its fatty acid chain. Clinical studies in patients with type 1 DM have demonstrated that when insulin detemir is administered twice a day, it has a smoother time–action profile and a reduced prevalence of hypoglycemia as compared with NPH insulin. Additional clinical studies are currently in progress with the aim of submitting insulin detemir for registration in the United States.

The wide variability in the kinetics of insulin action between and even within individuals must be emphasized. The time to peak hypoglycemic effect and insulin levels can vary by 50%. This variability is caused, at least in part, by large variations in the rate of subcutaneous absorption and often has been said to be more noticeable with the intermediate- and long-acting insulins. However, the administration of regular insulin can result in similar variability. When this variability is coupled with normal variations in diet and exercise, it is sometimes surprising how many patients do achieve good control of blood glucose concentrations.

Indications and Goals for Therapy. Subcutaneous administration of insulin is the primary treatment for all patients with type 1 DM, for patients with type 2 DM that is not controlled adequately by diet and/or oral hypoglycemic agents, and for patients with postpancreatectomy diabetes or gestational diabetes (American Diabetes Association, 1999). In addition, insulin is critical for the management of diabetic ketoacidosis, and it has an important role in the treatment of hyperglycemic, nonketotic coma and in the perioperative management of both type 1 and type 2 DM. In all cases, the goal is to normalize not only blood glucose but also all aspects of metabolism; the latter is difficult to achieve. Optimal treatment requires a coordinated approach to diet, exercise, and the administration of insulin. A brief overview of the principles of therapy is given below. (For a more detailed description, *see* LeRoith *et al.*, 2000.)

Near-normoglycemia can be attained in patients with multiple daily doses of insulin or with infusion pump therapy. The goal is to achieve a fasting blood glucose concentration of between 90 and 120 mg/dl (5 to 6.7 mM) and a 2-hour postprandial value below 150 mg/dl (8.3 mM). Goal hemoglobin A_{1C} values should be below 7% and are advocated by some authorities to be below 6.5%. In less disciplined patients, or in those with defective responses of counter-regulatory hormones, it may be necessary to accept higher fasting [*e.g.*, 140 mg/dl (7.8 mM)] and 2-hour postprandial [*e.g.*, 200 to 250 mg/dl (11.1 to 13.9 mM)] blood glucose concentrations.

Daily Requirements. Insulin production by a normal, thin, healthy person is between 18 and 40 units/day or about 0.2 to 0.5 units/kg of body weight per day. About half this amount is secreted in the basal state and about half in response to meals. Thus, basal secretion is about 0.5 to 1 units/h; after an oral glucose load, insulin secretion may increase to 6 units/h. In nondiabetic, obese, and insulin-resistant individuals, insulin secretion may be increased fourfold or more. Insulin is secreted into the portal circulation, and about 50% is destroyed by the liver before reaching the systemic circulation.

In a mixed population of type 1 DM patients, the average dose of insulin is usually 0.6 to 0.7 units/kg body weight per day, with a range of 0.2 to 1 units/kg per day. Obese patients generally require more (about 2 units/kg per day) because of resistance of peripheral tissues to insulin. Patients who require less insulin than 0.5 units/kg per day may have some endogenous production of insulin or may be more sensitive to the hormone because of good physical conditioning. As in nondiabetics, the daily requirement for insulin can be divided into basal and postprandial needs. The basal dose suppresses lipolysis, proteolysis, and hepatic glucose production; it is usually 40% to 60% of the total daily dose. The dose necessary for disposition of nutrients after meals usually is given before meals. Insulin often has been administered as a single daily dose of intermediate-acting insulin, alone or in combination with regular insulin. This is rarely sufficient to achieve true euglycemia; since hyperglycemia is the major determinant of long-term complications of diabetes, more complex regimens that include combinations of intermediate- or long-acting insulins with regular insulin are used to reach this goal.

A number of commonly used dosage regimens that include mixtures of insulin given in two or three daily injections are depicted in Figure 60–5 (LeRoith *et al.*, 2000). The most frequently used is the so-called split-mixed regimen involving the prebreakfast and presupper injection of a mixture of regular and intermediate-acting insulins (Figure 60–5A). When the presupper NPH or lente insulin is not sufficient to control hyperglycemia throughout the night, the evening dose may be divided into a presupper dose of regular insulin followed by NPH or lente insulin at bedtime (Figure 60–5B). Both normal and diabetic individuals have an increased requirement for insulin in the early morning; this is termed the *dawn phenomenon* and makes the kinetics and timing of the evening dose of insulin extremely important.

An alternative regimen that is gaining widespread use involves multiple daily injections consisting of basal administration of long-acting insulin (*e.g.*, insulin glargine) either before breakfast or at bedtime and preprandial injections of a short-acting insulin (Figure 60–5C). This method is called *basal/bolus* and is very similar to the pattern of insulin administration achieved with a subcutaneous infusion pump (Figure 60–5E). Following the successful demonstration that intensive glycemic control can reduce the risk of micro- and macrovascular complications in patients with type 2 DM, there has been increased interest in using insulin earlier in the treatment of these patients. Data from the UKPDS indicate that 50% of relative β-cell insulin secretory capacity is lost for every 6 years of type 2 DM. This progressive insulin deficiency as type 2 DM progresses makes it increasingly difficult to achieve tight glycemic control (hemoglobin $A_{1C} < 7.0\%$) with oral antihyperglycemic agents. One way to improve control in this setting is to introduce basal-acting insulin in combination with oral hypoglycemic agents. The exact combination of therapies should be guided by the β-cell secretory reserve in each patient. Thus, in an individual with some exogenous insulin secretory capacity (*i.e.*, a measurable circulating C peptide level), combining an oral insulin secretagogue (*see* below) with basal insulin may provide smooth and efficient glycemic control. The addition of a second oral agent, such as an insulin sensitizer (*see* below), either alone or in combination with an oral insulin secretagogue, also may provide good therapeutic results. This combination allows the oral agents to provide postprandial glycemic control while the basal insulin provides the foundation for normalizing fasting or "basal" glucose levels.

In all patients, careful monitoring of therapeutic end points directs the insulin dose used. This approach is facilitated by the use of home glucose monitors and measurements of hemoglobin A_{1c}. Special care must be taken when the patient has other underlying diseases, deficiencies in other endocrine systems (*e.g.*, adrenocortical or pituitary failure), or substantial resistance to insulin.

Factors That Affect Insulin Absorption. The degree of control of plasma glucose may be modified by changes in insulin absorption, factors that alter insulin action, diet, exercise, and other factors. Factors that determine the rate of absorption of insulin after subcutaneous administration include the site of injection, the type of insulin, subcutaneous blood flow, smoking, regional muscular activity at the site of the injection, the volume and concentration of the injected insulin, and depth of injection (insulin has a more rapid onset of action if delivered intramuscularly rather than subcutaneously).

When insulin is injected subcutaneously, there can be an initial "lag phase" followed by a slow but steadily increasing rate of absorption. The initial lag phase almost disappears when a reduced concentration or volume of insulin is injected.

Insulin usually is injected into the subcutaneous tissues of the abdomen, buttock, anterior thigh, or dorsal arm. Absorption is usually most rapid from the abdominal wall, followed by the arm, buttock, and thigh. Rotation of insulin injection sites traditionally has been advocated to avoid lipohypertrophy or lipoatrophy, although these conditions are less likely to occur with highly purified preparations of insulin. If a patient is willing to inject into the abdomen, injections can be rotated

Figure 60–5
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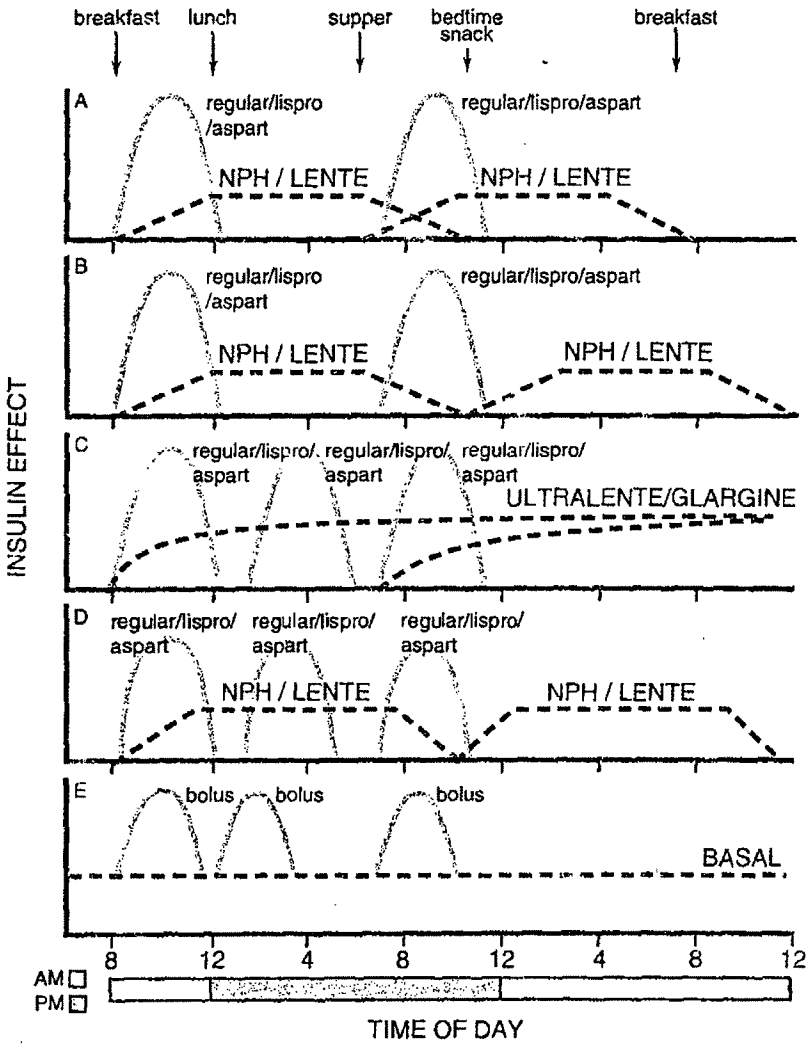


Figure 60–5. ***Common multidose insulin regimens.*** *A.* Typical split-mixed regimen consisting of twice-daily injections of a mixture of regular (regular/lispro/aspart) and intermediate-acting (NPH or lente) insulin. *B.* A variation in which the evening dose of intermediate-acting insulin is delayed until bedtime to increase the amount of insulin available the next morning. *C.* A regimen that incorporates ultralente or glargine insulin. *D.* A variation that includes premeal short-acting insulin with intermediate-acting insulin at breakfast and bedtime. *E.* Patterns of insulin administration with a regimen of continuous subcutaneous insulin infusion.

throughout the entire area, thereby eliminating the injection site as a cause of variability in the rate of absorption. The abdomen currently is the preferred site of injection in the morning because insulin is absorbed about 20% to 30% faster from that site than from the arm. If the patient refuses to inject into the abdominal area, it is preferable to select a consistent injection site for each component of insulin treatment (*e.g.*, prebreakfast dose into the thigh, evening dose into the arm).

Several other factors may affect the absorption of insulin. In a small group of patients, subcutaneous degradation of insulin has been observed, and this has necessitated the injection of large amounts of insulin for adequate metabolic control. Increased subcutaneous blood flow (brought about by massage, hot baths, or exercise) increases the rate of absorption. In the upright posture, subcutaneous blood flow diminishes considerably in the legs and to a lesser extent in the abdominal

wall. An altered volume or concentration of injected insulin affects the rate of absorption and the duration of action. When regular insulin is mixed with lente insulin, some of the regular insulin becomes modified, causing a partial loss of the rapidly acting component. This problem is even more severe if regular insulin is mixed with ultralente insulin. Injections of mixtures of insulin preparations thus should be made without delay. There is less delay in absorption of regular insulin when it is mixed with NPH insulin. Stable, mixed combinations of NPH and regular insulin in proportions of 50:50, 60:40, 70:30, and 80:20, respectively, are available commercially; in the United States, only the 70:30 and 50:50 combinations are available. Combinations of lispro protamine-Lispro (75/25, HUMALOG MIX) and aspart protamine-aspart (70/30, NOVOLOG MIX) are also available in the United States (Table 60–4). "Pen devices" containing prefilled regular, lispro, NPH, glargine, or premixed regular-NPH, lispro protamine-lispro, or apsart protamine-aspart combinations have proven to be popular with many diabetic patients.

Jet injector systems that enable patients to receive subcutaneous insulin "injections" without a needle are available. These devices are rather expensive and cumbersome but are preferred by some patients. Dispersal of insulin throughout an area of subcutaneous tissue theoretically should increase the rate of absorption of both regular and intermediate-acting insulins; however, this result has not always been observed.

Subcutaneous insulin administration results in anti-insulin IgG antibody formation. Older, impure preparations of animal insulins were far more antigenic than the more recent purified porcine and recombinant human preparations. It is disputed whether chronic therapy with human insulin reduces antibody production compared with monocomponent porcine insulin. Regardless, it is clear that human insulin is immunogenic. In the vast majority of patients receiving insulin treatment, circulating anti-insulin antibodies do not alter the pharmacokinetics of the injected hormone.

In rare patients who have a high titers of anti-insulin antibodies, the kinetics of action of regular insulin may resemble those of an intermediate-acting insulin, which itself may become longer acting. Such effects could lead to increased postprandial hyperglycemia (owing to decreased action of regular insulin) but nighttime hypoglycemia (owing to the prolonged action of intermediate insulin).

IgG antibodies can cross the placenta, raising the possibility that anti-insulin antibodies could cause fetal hyperglycemia by neutralizing fetal insulin. On the other hand, fetal or neonatal hypoglycemia could result from the undesirable and unpredictable release of insulin from insulin–antibody complexes. Switching from bovine/porcine to monocomponent insulin preparations has been shown to reduce anti-insulin antibodies, leading to the recommendation that only human insulin be used during pregnancy.

Continuous Subcutaneous Insulin Infusion. A number of pumps are available for continuous subcutaneous insulin infusion (CSII) therapy. CSII, or "pump," therapy is not suitable for all patients because it demands considerable attention, especially during the initial phases of treatment. However, for patients interested in intensive insulin therapy, a pump may be an attractive alternative to several daily injections. Most modern pumps provide a constant basal infusion of insulin and have the option of different infusion rates during the day and night to help avoid the dawn phenomenon and bolus injections that are programmed according to the size and nature of a meal.

Pump therapy presents some unique problems. Since all the insulin used is short acting and there is a minimal amount of insulin in the subcutaneous pool at any given time, insulin deficiency and ketoacidosis may develop rapidly if therapy is interrupted accidentally. Although modern pumps have warning devices that detect changes in line pressure, mechanical problems such as pump failure, dislodgement of the needle, aggregation of insulin in the infusion line, or accidental kinking of the infusion catheter may occur. There also is a possibility of subcutaneous abscesses and cellulitis. Selection of the most appropriate patients is extremely important for success with pump therapy. Offsetting these potential problems, pump therapy is capable of producing a more physiological profile of insulin replacement during exercise (where insulin production is decreased) and therefore less hypoglycemia than do traditional subcutaneous insulin injections.

Adverse Reactions. Hypoglycemia. The most common adverse reaction to insulin is hypoglycemia. This may result from an inappropriately large dose, from a mismatch between the time of peak delivery of insulin and food intake, or from superimposition of additional factors that increase sensitivity to insulin (*e.g.*, adrenal or pituitary insufficiency) or that increase insulin-independent glucose uptake (*e.g.*, exercise). The more vigorous the attempt to achieve euglycemia, the more frequent are the episodes of hypoglycemia. In the DCCT, the incidence of severe hypoglycemic reactions was three times higher in the intensive insulin therapy group than in the conventional therapy group (DCCT Research Group, 1993). Milder but significant hypoglycemic episodes were much more common than were severe reactions, and their frequency also increased with intensive therapy. Hypoglycemia is the major risk that always must be weighed against benefits of intensive therapy.

There is a hierarchy of physiological responses to hypoglycemia. The first response is a reduction of endogenous insulin secretion, which occurs at a plasma

glucose level of about 70 mg/dl (3.9 mM); thereafter, the counter-regulatory hormones—epinephrine, glucagon, growth hormone, cortisol, and norepinephrine—are released. Symptoms of hypoglycemia are first discerned at a plasma glucose level of 60 to 80 mg/dl (3.3 to 4.4 mM). Sweating, hunger, paresthesias, palpitations, tremor, and anxiety, principally of autonomic origin, usually are seen first. Difficulty in concentrating, confusion, weakness, drowsiness, a feeling of warmth, dizziness, blurred vision, and loss of consciousness (*i.e., the neuroglycopenic symptoms*) usually occur at lower plasma glucose levels than do autonomic symptoms. In a normal individual, plasma glucose levels are tightly regulated, and it is only under rare conditions that hypoglycemia occurs.

Glucagon and epinephrine are the predominant counter-regulatory hormones in acute hypoglycemia in newly diagnosed type 1 DM patients and normal subjects. In patients with type 1 DM of longer duration, the glucagon secretory response to hypoglycemia becomes deficient, but effective glucose counter-regulation still occurs because epinephrine plays a compensatory role. Patients with type 1 DM thus become dependent on epinephrine for counter-regulation, and if this mechanism becomes deficient, the incidence of severe hypoglycemia increases. This occurs in patients with diabetes of long duration who have autonomic neuropathy. The absence of both glucagon and epinephrine can lead to prolonged hypoglycemia, particularly during the night, when some individuals can have extremely low plasma glucose levels for several hours. Severe hypoglycemia can lead to convulsions and coma.

In addition to autonomic neuropathy, several related syndromes of defective counter-regulation contribute to the increased incidence of severe hypoglycemia in intensively treated type 1 DM patients. These include hypoglycemic unawareness, altered thresholds for release of counter-regulatory hormones, and deficient secretion of counter-regulatory hormones (reviewed by Cryer 1993).

With the ready availability of home glucose monitoring, hypoglycemia can be documented in most patients who experience suggestive symptoms. Hypoglycemia that occurs during sleep may be difficult to detect but should be suspected from a history of morning headaches, night sweats, or symptoms of hypothermia. Nocturnal hypoglycemia has been proposed as a cause of morning hyperglycemia in type 1 DM patients. This syndrome, known as the *Somogyi phenomenon*, was reputedly due to an elevation of counter-regulatory hormones in response to nocturnal hypoglycemia, but several groups of investigators have been unable to reproduce it. Moreover, neuroendocrine counter-regulatory responses are severely diminished with disease duration and intensive control. Therefore, it is unlikely that in patients with reduced neuroendocrine responses to hypoglycemia, nocturnal counter-regulatory responses to hypoglycemia could be responsible for morning hyperglycemia. The practice of reducing nighttime insulin doses in type 1 DM subjects with morning hyperglycemia thus cannot now be recommended. Rather, the current recommended therapeutic approach to treating morning hyperglycemia is to administer more long- or intermediate-acting insulin the night before, perhaps at bedtime, or to increase the basal rate of a CSII pump between the hours of 3 and 7 A.M.

All diabetic patients who receive insulin should be aware of the symptoms of hypoglycemia, carry some form of easily ingested glucose, and carry an identification card or bracelet containing pertinent medical information. When possible, patients who suspect that they are experiencing hypoglycemia should document the glucose concentration with a measurement. Mild-to-moderate hypoglycemia may be treated simply by ingestion of glucose. When hypoglycemia is severe, it should be treated with intravenous glucose or an injection of glucagon (*see* below).

Insulin Allergy and Resistance. Although there has been a dramatic decrease in the incidence of resistance and allergic reactions to insulin with the use of recombinant human insulin or highly purified preparations of the hormone, these reactions still occur as a result of reactions to the small amounts of aggregated or denatured insulin in all preparations, to minor contaminants, or because of sensitivity to one of the components added to insulin in its formulation (protamine, Zn^{2+}, phenol, *etc.*). The most frequent allergic manifestations are IgE-mediated local cutaneous reactions, although on rare occasions patients may develop life-threatening systemic responses or insulin resistance owing to IgG antibodies. Attempts should be made to identify the underlying cause of the hypersensitivity response by measuring insulin-specific IgG and IgE antibodies. Skin testing also is useful; however, many patients exhibit positive reactions to intradermal insulin without experiencing any adverse effects from subcutaneous insulin. If patients have allergic reactions to porcine insulin, human insulin should be used. If allergy persists, desensitization may be attempted; it is successful in about 50% of cases. Antihistamines may provide relief in patients with cutaneous reactions, whereas glucocorticoids have been used in patients with resistance to insulin or more severe systemic reactions.

Lipoatrophy and Lipohypertrophy. Atrophy of subcutaneous fat at the site of insulin injection (lipoatrophy) is probably a variant of an immune response to insulin, whereas lipohypertrophy (enlargement of subcutaneous fat depots) has been ascribed to the lipogenic action of high local concentrations of insulin (LeRoith *et al.*, 2000). Both problems may be related to some contaminant in insulin and are rare with more purified preparations. However, hypertrophy occurs frequently with human insulins if patients inject themselves repeatedly in the same site. When these problems occur, they may cause irregular absorption of insulin, as well as a cosmetic problem. The recommended treatment is to avoid the hypertrophic areas by using other injection sites and to inject insulin into the periphery of the atrophic sites in an attempt to restore the subcutaneous adipose tissue.

Insulin Edema. Some degree of edema, abdominal bloating, and blurred vision develops in many diabetic patients with severe hyperglycemia or ketoacidosis that is brought under control with insulin. This is associated with a weight gain of 0.5 to 2.5 kg. The edema usually disappears spontaneously within several days to a week unless there is underlying cardiac or renal disease. Edema is attributed primarily to retention of Na^+, although increased capil-

lary permeability associated with inadequate metabolic control also may contribute.

Insulin Treatment of Ketoacidosis and Other Special Situations. Acutely ill diabetic patients may have metabolic disturbances that are sufficiently severe or labile to justify intravenous administration of insulin. Such treatment is most appropriate in patients with ketoacidosis. Although there has been some controversy over appropriate dosage, infusion of a relatively low dose of insulin (0.1 units/kg per hour) will produce plasma concentrations of insulin of about 100 μunits/ml—a level sufficient to inhibit lipolysis and gluconeogenesis completely and to produce near-maximal stimulation of glucose uptake in normal individuals. In most patients with diabetic ketoacidosis, blood glucose concentrations will fall by about 10% per hour; the acidosis is corrected more slowly. As treatment proceeds, it often is necessary to administer glucose along with the insulin to prevent hypoglycemia but to allow clearance of all ketones. Some physicians prefer to initiate therapy with a loading dose of insulin, but this tactic appears unnecessary because steady-state concentrations of the hormone are achieved within 30 minutes with a constant infusion. Patients with nonketotic hyperglycemic coma typically are more sensitive to insulin than are those with ketoacidosis. Appropriate replacement of fluid and electrolytes is an integral part of the therapy in both situations because there is always a major deficit. Regardless of the exact insulin regimen, the key to effective therapy is careful and frequent monitoring of the patient's clinical status, glucose, and electrolytes. A frequent error in the management of such patients is the failure to administer insulin subcutaneously at least 30 minutes before intravenous therapy is discontinued. This is necessary because of the very short half-life of insulin.

Intravenous administration of insulin also is well suited to the treatment of diabetic patients during the perioperative period and during childbirth (Jacober and Sower, 1999). There is debate, however, about the optimal route of insulin administration during surgery. Although some clinicians advocate subcutaneous insulin administration, most recommend intravenous insulin infusion. The two most widely used protocols for intravenous insulin administration are the variable-rate regimen and the glucose–insulin–potassium (GIK) infusion method (*see* LeRoith *et al.*, 2000). Both protocols provide stable plasma glucose, fluid, and electrolyte levels during the operative and postoperative period. Despite this, many physicians give patients half their normal daily dose of insulin as intermediate-acting insulin subcutaneously on the morning of an operation and then administer 5% dextrose infusions during surgery to maintain glucose concentrations. This approach provides less minute-to-minute control than is possible with intravenous regimens and also may increase the likelihood of hypoglycemia. Newer subcutaneously administered basal and rapid-acting analogs may provide smoother glycemic control without the drawback of hypoglycemia. Recent multicenter trials have demonstrated dramatic improvement in patient outcome, including significant reductions in mortality, when intensive insulin regimens (predominantly intravenous) have been used to reduce glycemia after myocardial infarction or surgery (Malmberg *et al.*, 1995; Davies and Lawrence, 2002; van den Berghe *et al.*, 2001; van den Berghe 2004).

Drug Interactions and Glucose Metabolism. A large number of drugs can cause hypoglycemia or hyperglycemia or may alter the response of diabetic patients to their existing therapeutic regimens. Some drugs with hypoglycemic or hyperglycemic effects and their presumed sites of action are listed in Table 60–5.

Aside from insulin and oral hypoglycemic drugs, the most common drug-induced hypoglycemic states are those caused by *ethanol, β adrenergic receptor antagonists,* and *salicylates.* The primary action of ethanol is to inhibit gluconeogenesis. This is not an idiosyncratic reaction but is observed in all individuals. In diabetic patients, β adrenergic receptor antagonists pose a risk of hypoglycemia because of their capacity to inhibit the effects of catecholamines on gluconeogenesis and glycogenolysis. These agents also may mask the sympathetically mediated symptoms associated with the fall in blood glucose (*e.g.*, tremor and palpitations). Salicylates, on the other hand, exert their hypoglycemic effect by enhancing pancreatic β-cell sensitivity to glucose and potentiating insulin secretion. These agents also have a weak insulinlike action in the periphery. *Pentamidine,* an antiprotozoal agent used for the treatment of infections caused by *Pneumocystis carinii,* apparently can cause both hypoglycemia and hyperglycemia. The hypoglycemic effect results from destruction of β cells and release of insulin; continued use may cause secondary hypoinsulinemia and hyperglycemia. A number of drugs have no direct hypoglycemic action but may potentiate the actions of sulfonylureas (*see* below).

An equally large number of drugs may cause hyperglycemia in normal individuals or impair metabolic control in diabetic patients. Many of these agents have direct effects on peripheral tissues that counter the actions of insulin; examples include epinephrine, glucocorticoids, atypical antipsychotic drugs such as *clozapine* and *olanzapine,* and drugs used in highly active antiretroviral therapy (HAART) of HIV-1 infection (especially the protease inhibitors). Other drugs cause hyperglycemia by inhibiting insulin secretion directly (*e.g., phenytoin, clonidine,* and Ca^{2+}-channel blockers) or indirectly *via* depletion of K^+ (diuretics). It is important to be aware of such interactions and to modify treatment regimens for diabetic patients accordingly.

New Forms of Insulin Therapy. There are a number of experimental approaches to insulin delivery, including the use of new insulins, new routes of administration, intraperitoneal delivery devices, implantable pellets, the closed-loop artificial pancreas, islet cell and pancreatic transplantation, and gene therapy.

New Routes of Delivery. Attempts have been made to administer insulin orally, nasally, rectally, by inhalation, and by subcutaneous implantation of pellets. The most promising of these alternatives is by inhalation, which can be achieved by addition of various adjuvants such as *mannitol, glycine,* and *sodium citrate* to insulin to increase its absorption through the pulmonary mucosa (Skyler *et al.*, 2001; Cefalu *et al.*, 2001). Absorption is rapid and approaches the

Table 60–5
Some Drugs That Cause Hypoglycemia or Hyperglycemia

DRUG	POSSIBLE SITE OF ACTION			
	Pancreas	*Liver*	*Periphery*	*Other*
Drugs with Hypoglycemic Effects				
β Adrenergic receptor antagonists		+	+	+
Salicylates	+			
Indomethacin*				
Naproxen*				
Ethanol		+		
Clofibrate				+
Angiotensin-converting enzyme inhibitors			+	
Li^+			+	
Theophylline	+	+	+	
Ca^{2+}	+			
Bromocriptine				
Mebendazole			+	
Sulfonamides	+			
Sulbactam–ampicillin*				+
Tetracycline*				
Pyridoxine				
Pentamidine†		+		
Drugs with Hyperglycemic Effects	+			
Epinephrine	+	+	+	
Glucocorticoids		+	+	
Diuretics	+		+	
Atypical antipsychotics‡			+	
HIV-1 protease inhibitors§			+	+
Diazoxide	+			
β_2 Adrenergic receptor agonists	+	+	+	
Ca^{2+}-channel blockers	+			
Phenytoin	+			
Clonidine	+			
H_2-receptor blockers	+			+
Pentamidine†				
Morphine	+			+
Heparin				
Nalidixic acid				+
Sulfinpyrazone*				?
Marijuana				
Nicotine*				+

*Although these drugs are reported to have an effect on control of diabetes, there are no conclusive data about their effects on carbohydrate metabolism. †Short-term effect is insulin release and hypoglycemia. ‡Atypical antipsychotics: clozapine, olanzapine, risperidone. §HIV-1 protease inhibitors: ritonavir, lopinavir, aprenavir, nelfinavir, indinavir, saquinavir. SOURCE: Adapted from Koffler *et al.*, 1989, with permission.

rate achieved with subcutaneous administration of regular insulin. Further work is under way with the aim of reducing the size and increasing the convenience of the inhaled delivery systems. Implantable pellets have been designed to release insulin slowly over days or weeks. Although oral delivery of insulin would be preferred by patients and would provide higher relative concentrations of insulin in the portal circulation, attempts to increase intestinal absorption of the hormone have met with only limited success. Efforts have focused on protection of insulin by encapsulation or incorporation into liposomes. Intraperitoneal infusion of insulin into the portal circulation has been used experimentally in human subjects for periods of several months.

Transplantation and Gene Therapy. Transplantation and gene therapy are provocative approaches to insulin replacement. Segmental pancreatic transplantation has been employed successfully in hundreds of patients (Sutherland *et al.*, 2004). However, the surgery is technically complex and usually is considered only in patients with advanced disease and complications. The best-documented benefits have been in patients who also require a kidney transplant for diabetic nephropathy. Islet cell transplants theoretically are less complicated. Successful protocols for islet cell transplants were based on advances in islet preparation and a novel glucocorticoid-free immunosuppressive regimen (Robertson, 2004). The precise role of islet cell transplantation is debated, and the supply of available islet preparations remains very limited. In rodents, gene therapy using transcription factors that regulate β-cell function has been used to transdifferentiate hepatocytes into a functional endocrine pancreas, eliminating the need for insulin therapy for months in experimental models of diabetes mellitus (Meivar-Levy and Ferber, 2004).

ORAL HYPOGLYCEMIC AGENTS

History. In contrast to the systematic studies that led to the isolation of insulin, the *sulfonylureas* were discovered accidentally. In 1942, Janbon and colleagues noted that some sulfonamides caused hypoglycemia in experimental animals. Soon thereafter, *1-butyl-3-sulfonylurea (carbutamide)* became the first clinically useful sulfonylurea for the treatment of diabetes. Although later withdrawn because of adverse effects on the bone marrow, this compound led to the development of the entire class of sulfonylureas. Clinical trials of *tolbutamide*, the first widely used member of this group, were instituted in patients with type 2 DM in the early 1950s. Since that time, approximately 20 different agents of this class have been in use worldwide.

In 1997, *repaglinide*, the first member of a new class of oral insulin secretagogues called *meglitinides* (benzoic acid derivatives), was approved for clinical use; this agent has gained acceptance as a fast-acting premeal therapy to limit postprandial hyperglycemia.

The goat's rue plant (*Galega officinalis*), used to treat diabetes in Europe in medieval times, was found in the early twentieth century to contain *guanadine*. Guanadine has hypoglycemic properties but was too toxic for clinical use. During the 1920s, *biguanides* were investigated for use in diabetes, but they were overshadowed by the discovery of insulin. Later, the antimalarial agent *chloroguanide* was found to have weak hypoglycemic action. Shortly after the introduction of the sulfonylureas, the first biguanides became available for clinical use. However, *phenformin*, the primary drug in this group, was withdrawn from the market in the United States and Europe because of an increased frequency of lactic acidosis associated with its use. Another biguanide, metformin, has been used extensively in Europe without significant adverse effects and was approved for use in the United States in 1995.

Thiazolidinediones were introduced in 1997 as the second major class of "insulin sensitizers." These agents bind to peroxisome proliferator–activated receptors (principally PPARγ), resulting in increased glucose uptake in muscle and reduced endogenous glucose production. The first of these agents, *troglitazone*, was withdrawn from use in the United States in 2000 because of an association with hepatic toxicity. Two other agents of this class, *rosiglitazone* and *pioglitazone*, have not been associated with widespread liver toxicity and are used worldwide.

Sulfonylureas

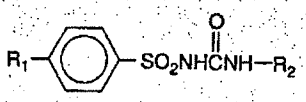
Chemistry. The sulfonylureas are divided into two groups or generations of agents. Their structural relationships are shown in Table 60–6. All members of this class of drugs are substituted arylsulfonylureas. They differ by substitutions at the *para* position on the benzene ring and at one nitrogen residue of the urea moiety. The first group of sulfonylureas includes tolbutamide, *acetohexamide*, *tolazamide*, and *chlorpropamide*. A second, more potent generation of hypoglycemic sulfonylureas has emerged, including *glyburide (glibenclamide)*, *glipizide*, *gliclazide*, and *glimepiride*.

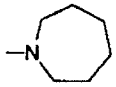
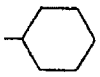
Mechanism of Action. Sulfonylureas cause hypoglycemia by stimulating insulin release from pancreatic β cells. Their effects in the treatment of diabetes, however, are more complex. The acute administration of sulfonylureas to type 2 DM patients increases insulin release from the pancreas. Sulfonylureas also may further increase insulin levels by reducing hepatic clearance of the hormone. In the initial months of sulfonylurea treatment, fasting plasma insulin levels and insulin responses to oral glucose challenges are increased. With chronic administration, circulating insulin levels decline to those that existed before treatment, but despite this reduction in insulin levels, reduced plasma glucose levels are maintained. The explanation for this is not clear, but it may relate to reduced plasma glucose allowing circulating insulin to have more pronounced effects on its target tissues and to the fact that chronic hyperglycemia *per se* impairs insulin secretion (glucose toxicity).

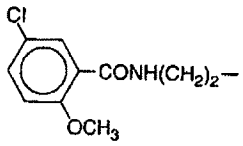
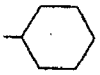
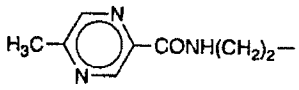
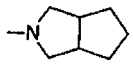
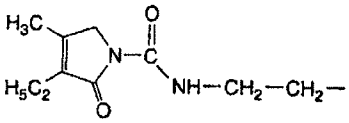
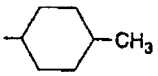
The absence of acute stimulatory effects of sulfonylureas on insulin secretion during chronic treatment is attributed to down-regulation of cell surface receptors for sulfonylureas on the pancreatic β cell. If chronic sulfonylurea therapy is discontinued, pancreatic β-cell response to acute administration of the drug is restored. Sulfonylureas also stimulate release of somatostatin, and they may suppress the secretion of glucagon slightly.

Table 60–6
Structural Formulas of the Sulfonylureas

GENERAL FORMULA:



First-Generation Agents	R_1	R_2
Tolbutamide (ORINASE, others)	H_3C-	$-C_4H_9$
Chlorpropamide (DIABINESE, others)	Cl—	$-C_3H_7$
Tolazamide (TOLINASE, others)	H_3C-	
Acetohexamide (DYMELOR, others)	H_3CCO-	

Second-Generation Agents	R_1	R_2
Glyburide (Glibenclamide, MICRONASE, DIABETA, others)		
Glipizide (GLUCOTROL, others)		
Gliclazide (DIAMICRON, others; unavailable in the U.S.)	H_3C-	
Glimepiride (AMARYL)		

Sulfonylureas bind to the SUR1 subunits and block the ATP-sensitive K^+ channel (Aguilar-Bryan *et al.*, 1995; Philipson and Steiner, 1995). The drugs thus resemble physiological secretagogues (*e.g.*, glucose, leucine), which also lower the conductance of this channel. Reduced K^+ conductance causes membrane depolarization and influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels.

There has been controversy about whether or not sulfonylureas have clinically significant extrapancreatic effects. In general, attempts to ascribe the long-term blood glucose-lowering effects of sulfonylureas to specific changes in insulin action on target tissues are confounded by the effects of a lower prevailing blood glucose level. Although extrapancreatic effects of sulfonylureas can be demonstrated, they are of minor clinical significance in the treatment of type 2 DM patients.

Absorption, Fate, and Excretion. The sulfonylureas have similar spectra of activities; thus their pharmacokinetic properties are their most distinctive characteristics (*see* Appendix II). Although the rates of absorption of the different sulfonylureas vary, all are effectively absorbed from the gastrointestinal tract. However, food and hyperglycemia can reduce the absorption of sulfonylureas. Hyperglycemia *per se* inhibits gastric and intestinal motility and thus can retard the absorption of many drugs. In view of the time required to reach an optimal concentration in plasma, sulfonylureas with short half-lives may be more effective when given 30 minutes before eating. Sulfonylureas in plasma are largely (90% to 99%) bound to protein, especially albumin; plasma protein binding is least for chlorpropamide and greatest for glyburide. The volumes of distribution of most of the sulfonylureas are about 0.2 L/kg.

The first-generation sulfonylureas vary considerably in their half-lives and extents of metabolism. The half-life of acetohexamide is short, but the drug is reduced to an active compound whose half-life is similar to those of tolbutamide and tolazamide (4 to 7 hours). It may be necessary to take these drugs in divided daily doses. Chlorpropamide has a long half-life (24 to 48 hours). The second-generation agents are approximately 100 times more potent than are those in the first group. Although their half-lives are short (3 to 5 hours), their hypoglycemic effects are evident for 12 to 24 hours, and they often can be administered once daily. The reason for the discrepancies between their half-lives and duration of action is not clear.

All the sulfonylureas are metabolized by the liver, and the metabolites are excreted in the urine. Metabolism of chlorpropamide is incomplete, and about 20% of the drug is excreted unchanged. Thus sulfonylureas should be administered with caution to patients with either renal or hepatic insufficiency.

Adverse Reactions. Adverse effects of the sulfonylureas are infrequent, occurring in about 4% of patients taking first-generation drugs and perhaps slightly less often in patients receiving second-generation agents. Not unexpectedly, sulfonylureas may cause hypoglycemic reactions, including coma. This is a particular problem in elderly patients with impaired hepatic or renal function who are taking longer-acting sulfonylureas. Sulfonylureas can be ranked in order of decreasing risk of causing hypoglycemia. It used to be thought that longer-acting sulfonylureas resulted in a greater prevalence of hypoglycemia. That is certainly the case when comparing the older preparations such as chlorpropamide (long acting) against tolbutamide (short acting). However, more recent second-generation sulfonylureas have very differing incidences of causing hypoglycemia despite similar half-lives. Thus glyburide (glibenclamide) has been reported to result in hypoglycemia in up to 20% to 30% of users, whereas another long-acting sulfonylurea, glimepiride, results in hypoglycemia in only 2% to 4% of users. A modified long-acting version of glipizide also results in a lower hypoglycemia frequency relative to gliburide.

Recent studies have provided an insight into the physiological basis for the differing rates of hypoglycemia occurring with these long-acting sulfonylureas. As described earlier for insulin, the ability of the body to inhibit endogenous insulin secretion is central to the homeostatic defense against hypoglycemia. This glucose-dependent inhibition of insulin secretion during hypoglycemia occurs with glimepiride but not with glyburide. Additionally, the major anti-insulin counter-regulatory hormone glucagon appears to be reduced by glyburide during hypoglycemia but is preserved during glimepiride therapy.

Severe hypoglycemia in the elderly can present as an acute neurological emergency that may mimic a cerebrovascular accident. Thus, it is important to check the plasma glucose level of any elderly patient presenting with acute neurological symptoms. Because of the long half-life of some sulfonylureas, it may be necessary to treat elderly hypoglycemic patients for 24 to 48 hours with an intravenous glucose infusion.

Many other drugs may potentiate the effects of the sulfonylureas, particularly the first-generation agents, by inhibiting their metabolism or excretion. Some drugs also displace the sulfonylureas from binding proteins, thereby transiently increasing the free concentration. These include other sulfonamides, *clofibrate,* and salicylates. Other drugs, especially ethanol, may enhance the action of sulfonylureas by causing hypoglycemia.

Other side effects of sulfonylureas include nausea and vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, and dermatological reactions. About 10% to 15% of patients who receive these drugs, particularly chlorpropa-

mide, develop an alcohol-induced flush similar to that caused by *disulfiram* (*see* Chapter 23). Sulfonylureas, especially chlorpropamide, also may induce hyponatremia by potentiating the effects of antidiuretic hormone on the renal collecting duct (*see* Chapter 29). This undesirable side effect occurs in up to 5% of all patients; it is less frequent with glyburide, glipizide, and glimepiride. This effect on water retention has been used to therapeutic advantage in patients with mild forms of central diabetes insipidus.

A long-running debate centered on whether treatment with sulfonylureas is associated with increased cardiovascular mortality; this possibility was suggested by a large multicenter trial [the University Group Diabetes Program (UGDP)]. The UGDP was designed to compare the effect of diet, oral agents (tolbutamide or phenformin), and fixed-dose insulin therapy on the development of vascular complications in type 2 DM. During an 8-year period of observation, patients who received tolbutamide had a twofold higher rate of cardiovascular death than patients treated with placebo or insulin (Meinert *et al.*, 1970). A 10-year debate followed on the validity of this conclusion because the observation was unexpected, the study had not been designed to test this question, and all the excess mortality occurred in only three centers. The recent UKPDS (U.K. Prospective Diabetes Study Group, 1998a) clearly demonstrated no excess cardiovascular mortality over a 14-year period in patients receiving first- or second-generation sulfonylureas. It is worth noting that some of the newer sulfonylurea agents may confer even greater cardiovascular benefits compared with earlier second-generation compounds. Glimepiride, the most recent sulfonylurea, exerts beneficial effects with regard to ischemic preconditioning as compared with glyburide. The physiological response to an ischemic event in the coronary vasculature is a reflex vasodilation to a subsequent ischemic episode. This reflex appears to be preserved with glimepiride but reduced with glyburide.

Therapeutic Uses. Sulfonylureas are used to control hyperglycemia in type 2 DM patients who cannot achieve appropriate control with changes in diet alone. In all patients, continued dietary restrictions are essential to maximize the efficacy of the sulfonylureas. Contraindications to the use of these drugs include type 1 DM, pregnancy, lactation, and for the older preparations, significant hepatic or renal insufficiency.

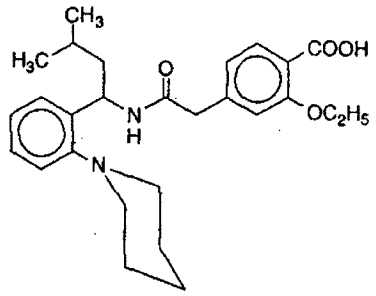
Between 50% and 80% of properly selected patients will respond initially to an oral hypoglycemic agent. All the drugs appear to be equally efficacious. Concentrations of glucose often are lowered sufficiently to relieve symptoms of hyperglycemia but may not reach normal levels. To the extent that complications of diabetes are related to hyperglycemia, the goal of treatment should be normalization of both fasting and postprandial glucose concentrations. About 5% to 10% of patients per year who respond initially to a sulfonylurea become secondary failures, as defined by unacceptable levels of hyperglycemia. This may occur as a result of a change in drug metabolism, progression of β-cell failure, change in dietary compliance, or misdiagnosis of a patient with slow-onset type 1 DM. Additional oral agent(s) can produce a satisfactory response, but most of these patients eventually will require insulin.

The usual initial daily dose of tolbutamide is 500 mg, and 3000 mg is the maximally effective total dose. Tolazamide and chlorpropamide usually are initiated in a daily dose of 100 to 250 mg, with maximal doses of 1000 (tolazamide) or 750 mg (chlorpropamide). Tolbutamide and tolazamide often are taken twice daily 30 minutes before breakfast and dinner. The initial daily dose of glyburide is 2.5 to 5 mg, and daily doses of more than 20 mg are not recommended. Therapy with glipizide usually is initiated with 5 mg given once daily. The maximal recommended daily dose is 40 mg; daily doses of more than 15 mg should be divided. The starting dose of gliclazide is 40 to 80 mg/day, and the maximal daily dose is 320 mg. Glimepiride therapy can begin with doses as low as 0.5 mg once per day. The maximal effective daily dose of the agent is 8 mg. Treatment with the sulfonylureas must be guided by the patient's response, which must be monitored frequently.

Combinations of insulin and sulfonylureas have been used in some patients with type 1 and type 2 DM. Studies in type 1 DM patients have provided no evidence that glucose control is improved by combination therapy. The results in type 2 DM patients have shown significant improvements in metabolic control. A prerequisite for a beneficial effect of combination therapy is residual β-cell activity; a short duration of diabetes also may predict a good response.

Repaglinide

Repaglinide (PRANDIN) is an oral insulin secretagogue of the meglitinide class. This agent is a derivative of benzoic acid, and its structure (shown below) is unrelated to that of the sulfonylureas.



REPAGLINIDE

Like sulfonylureas, repaglinide stimulates insulin release by closing ATP-dependent potassium channels in pancreatic β cells. The drug is absorbed rapidly from the gastrointestinal tract, and peak blood levels are obtained within 1 hour. The half-life of the drug is about 1 hour. These features of the drug allow for multiple preprandial use as compared with the classical once- or twice-daily

dosing of sulfonylureas. Repaglinide is metabolized primarily by the liver to inactive derivatives. Repaglinide should be used cautiously in patients with hepatic insufficiency. Because a small proportion (about 10%) of repaglinide is metabolized by the kidney, increased dosing of the drug in patients with renal insufficiency also should be performed cautiously. As with sulfonylureas, the major side effect of repaglinide is hypoglycemia.

Nateglinide

Nateglinide (STARLIX) is an orally effective insulin secretagogue derived from D-phenylalanine. Like sulfonylureas and repaglinide, nateglinide stimulates insulin secretion by blocking ATP-sensitive potassium channels in pancreatic β cells. Nateglinide promotes a more rapid but less sustained secretion of insulin than do other available oral antidiabetic agents (Kalbag *et al.*, 2001). The drug's major therapeutic effect is reducing postprandial glycemic elevations in type 2 DM patients. Nateglinide is approved by the FDA for use in type 2 DM and is most effective if administered in a dose of 120 mg 1 to 10 minutes before a meal. Nateglinide is metabolized primarily by the liver and thus should be used cautiously in patients with hepatic insufficiency. About 16% of an administered dose is excreted by the kidney as unchanged drug. Dosage adjustment is unnecessary in renal failure. Nateglinide therapy may produce fewer episodes of hypoglycemia than most other currently available oral insulin secretagogues including repaglinide (Horton *et al.*, 2001).

Biguanides

Metformin (GLUCOPHAGE, others) and phenformin were introduced in 1957, and *buformin* was introduced in 1958. The latter was of limited use, but metformin and phenformin were used widely. Phenformin was withdrawn in many countries during the 1970s because of an association with lactic acidosis. Metformin has been associated only rarely with that complication and has been used widely in Europe and Canada; it became available in the United States in 1995. Metformin given alone or in combination with a sulfonylurea improves glycemic control and lipid concentrations in patients who respond poorly to diet or to a sulfonylurea alone (DeFronzo *et al.*, 1995).

Mechanism of Action. Metformin is antihyperglycemic, not hypoglycemic (*see* Bailey, 1992). It does not cause insulin release from the pancreas and generally does not cause hypoglycemia, even in large doses. Metformin has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin. Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. At a molecular level, these actions are mediated at least in part by activation of the cellular kinase AMP-activated protein kinase (AMP kinase) (*see* below and Zhou *et al.*, 2001). The mechanism by which metformin reduces hepatic glucose production is controversial, but most data indicate an effect on reducing gluconeogenesis (Stumvoll *et al.*, 1995). Metformin also may decrease plasma glucose by reducing the absorption of glucose from the intestine, but this action has not been shown to have clinical relevance.

Absorption, Excretion, and Dosing. Metformin is absorbed mainly from the small intestine. The drug is stable, does not bind to plasma proteins, and is excreted unchanged in the urine. It has a half-life of about 2 hours. The maximum recommended daily dose of metformin in the United States is 2.5 g given in three doses with meals.

Precautions and Adverse Effects. Patients with renal impairment should not receive metformin. Other contraindications include hepatic disease, a past history of lactic acidosis (of any cause), cardiac failure requiring pharmacological therapy, or chronic hypoxic lung disease. The drug also should be discontinued temporarily prior to the administration of intravenous *contrast media* and prior to any surgical procedure. The drug should not be readministered any sooner than 48 hours after such procedures and should be withheld until renal function is determined to be normal. These conditions all predispose to increased lactate production and hence to the potentially fatal complication of lactic acidosis. The reported incidence of lactic acidosis during metformin treatment is less than 0.1 cases per 1000 patient-years, and the mortality risk is even lower.

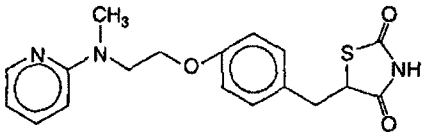
Acute side effects of metformin, which occur in up to 20% of patients, include diarrhea, abdominal discomfort, nausea, metallic taste, and anorexia. These usually can be minimized by increasing the dosage of the drug slowly and taking it with meals. Intestinal absorption of *vitamin* B_{12} and *folate* often is decreased during chronic metformin therapy, and calcium supplements reverse the effect of metformin on vitamin B_{12} absorption.

Consideration should be given to stopping treatment with metformin if the plasma lactate level exceeds 3 mM or in the setting of decreased renal or hepatic function. It also is prudent to stop metformin if a patient is undergoing a prolonged fast or is treated with a very low calorie diet. Myocardial infarction or septicemia mandates immediate drug discontinuation. Metformin usually is administered in divided doses two or three times daily. The maximum effective dose is 2.5 g/day. Metformin lowers hemoglobin A_{1c} values by about 2%, an effect comparable with that of the sulfonylureas. Metformin does not promote weight gain and can reduce plasma triglycerides by 15% to 20%. There is a strong consensus that reduction in hemoglobin A_{1c} by any therapy (insulin or

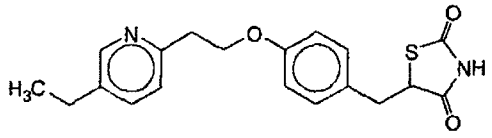
oral agents) diminishes microvascular complications. Metformin, however, is the only therapeutic agent that has been demonstrated to reduce macrovascular events in type 2 DM (U.K. Prospective Diabetes Study Group, 1998b). Metformin can be administered in combination with sulfonylureas, thiazolizinediones, and/or insulin. Fixed-dose combinations containing metformin and glyburide (GLUCOVANCE, others), glipizide (METAGLIP), and rosiglitazone (AVANDAMET) are available.

Thiazolidinediones

Three thiazolidinediones have been used in clinical practice (troglitazone, rosiglitazone, and pioglitazone); however, troglitazone was withdrawn from use because it was associated with severe hepatic toxicity. Rosiglitazone and pioglitazone can lower hemoglobin A_{1c} levels by 1% to 1.5% in patients with type 2 DM. These drugs can be combined with insulin or other classes of oral glucose-lowering agents. The thiazolidinediones tend to increase high-density lipoprotein (HDL) cholesterol but have variable effects on triglycerides and low-density lipoprotein (LDL) cholesterol. The structures of rosiglitazone and pioglitazone are:



ROSIGLITAZONE



PIOGLITAZONE

Mechanism of Action. Thiazolidinediones are selective agonists for nuclear peroxisome proliferator–activated receptor-γ (PPARγ). These drugs bind to PPARγ, which activates insulin-responsive genes that regulate carbohydrate and lipid metabolism. Thiazolidinediones require insulin to be present for their action. Thiazolidinediones exert their principal effects by increasing insulin sensitivity in peripheral tissue but also may lower glucose production by the liver. Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporters. The thiazolidinediones also can activate genes that regulate fatty acid metabolism in peripheral tissue. Although muscle is a major insulin-sensitive tissue, PPARγ is virtually absent in skeletal muscle. This has provoked questions as to how thiazolidinediones can reduce peripheral insulin resistance. One suggestion is that activation of PPARγ in adipose tissue reduces the flux of fatty acids into muscle, thereby lowering insulin resistance. Other suggestions include the activation of adipocyte hormones and/or adipokines, the most promising of which is adiponectin. Adiponectin is associated with increased insulin sensitivity and reportedly increases insulin sensitivity by elevating AMP kinase, which stimulates glucose transport into muscle and increases fatty acid oxidation (Havel, 2003). Because the actions of both metformin and the thiazolidinediones apparently converge on AMP kinase, it has emerged as an attractive target for drug development (Ruderman and Prentki, 2004).

Absorption, Excretion, and Dosing. Rosiglitazone (AVANDIA) and pioglitazone (ACTOS) are taken once a day. Both agents are absorbed within about 2 hours, but the maximum clinical effect is not observed for 6 to 12 weeks. The thiazolidinediones are metabolized by the liver and may be administered to patients with renal insufficiency but should not be used if there is active hepatic disease or significant elevations of serum liver transaminases.

Rosiglitazone is metabolized by hepatic cytochrome P450 (CYP) 2C8, whereas pioglitazone is metabolized by CYP3A4 and CYP2C8. As discussed in Chapter 3, other drugs that induce or inhibit these enzymes can cause drug interactions. Clinically significant interactions between the available thiazolidinediones and other drug classes have not yet been described, but further studies are in progress.

Precautions and Adverse Effects. Liver function should be monitored in patients receiving thiazolidinediones, even though pioglitazone and rosiglitazone rarely have been associated with hepatotoxicity (12 cases up to July 2004). This lower hepatotoxicity has been attributed to the lack of the tocopherol side chain that was included in the troglitazone molecule. Additionally, the rare cases of hepatotoxicity occurring with second-generation thiazolidinediones appear to be less severe than those occurring with troglitazone. Hepatotoxicity can occur several months after initiation of the drugs. Any patient who has suffered any hepatotoxicity (even abnormal liver function tests) while on a thiazolidinediones should not receive any drugs in this class. Thiazolidinediones also have been reported to cause anemia, weight gain, edema, and plasma volume expansion. Edema is more likely to occur when these agents are combined with insulin; these drugs should not be used in patients with New

York Heart Association class 3 or 4 heart failure. Fluid retention and even overt heart failure usually occur within 6 months of thiazolidinedione therapy. In most cases, the subjects had no past history of heart failure, but all had underlying abnormal cardiac function. Obese hypertensive individuals and those with cardiac diastolic dysfunction are at greatest risk for fluid retention with thiazolidinediones. Thiazolidinediones also can induce peripheral edema independent of heart failure; proposed mechanisms include an increase in weight, an expansion of plasma volume following a reduction in renal sodium excretion, or a direct effect to increase vascular permeability. Exacerbations of fluid retention and/or heart failure should be treated, and the thiazolidinedione should be discontinued.

The availability of thiazolidinediones as powerful PPARγ ligands has sparked a number of novel avenues of clinical research. Studies have investigated whether thiazolidinediones can improve insulin sensitivity in HIV-associated lipodystrophy (*see* Chapter 50). Studies also are underway to explore the effects of thiazolidinediones on nonalcoholic hepatic steatosis. Finally, small single-site studies have investigated whether rosiglitazone can slow the progression of atheromatous lesions in carotid and coronary arteries in both nondiabetic and type 2 DM patients. Results to date have been mixed, and further multicenter studies are ongoing.

α-Glucosidase Inhibitors

α-Glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α-glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates; the postprandial rise in plasma glucose is blunted in both normal and diabetic subjects.

α-Glucosidase inhibitors do not stimulate insulin release and therefore do not result in hypoglycemia. These agents may be considered as monotherapy in elderly patients or in patients with predominantly postprandial hyperglycemia. α-Glucosidase inhibitors typically are used in combination with other oral antidiabetic agents and/or insulin. The drugs should be administered at the start of a meal. They are poorly absorbed.

Acarbose (PRECOSE), an oligosaccharide of microbial origin, and *miglitol* (GLYSET), a desoxynojirimycin derivative, also competitively inhibit glucoamylase and sucrase but have weak effects on pancreatic α-amylase. They reduce postprandial plasma glucose levels in type 1 and type 2 DM subjects. α-Glucosidase inhibitors can significantly improve hemoglobin A_{1c} levels in severely hyperglycemic type 2 DM patients. However, in patients with mild-to-moderate hyperglycemia, the glucose-lowering potential of α-glucosidase inhibitors (assessed by hemoglobin A_{1c} levels) is about 30% to 50% of that of other oral antidiabetic agents.

α-Glucosidase inhibitors cause dose-related malabsorption, flatulence, diarrhea, and abdominal bloating. Titrating the dose of drug slowly (25 mg at the start of a meal for 4 to 8 weeks, followed by increases at 4- to 8-week intervals to a maximum of 75 mg before each meal) reduces gastrointestinal side effects. Smaller doses are given with snacks. Acarbose is most effective when given with a starchy, high-fiber diet with restricted amounts of glucose and sucrose. If hypoglycemia occurs when α-glucosidase inhibitors are used with insulin or an insulin secretagogue, glucose rather than sucrose, starch, or maltose should be administered.

Reduction in the Incidence of Type 2 DM

Type 2 DM is a rapidly expanding worldwide health problem. In addition, the number of individuals who have impaired glucose tolerance (often termed *prediabetes*) may be equal to or even higher than the number of people with diabetes. In the United States, nearly 20 million individuals are diagnosed with diabetes, but perhaps twice that number have *impaired glucose tolerance* (IGT), which is defined as a fasting plasma glucose concentration of between 100 and 126 mg/dl (5.6 to 7 mM) or 2-hour values in the oral glucose tolerance test of between 140 and 199 mg/dl (7.8 to 11 mM) (Expert Committee on the Diagnosis and Classification of Diabetes, 2003). The rate of progression of IGT to overt diabetes ranges from 9% to 15% worldwide. A major factor in this increased incidence of diabetes is obesity. In the United States, approximately 60% of the population is overweight or obese. Particularly troubling is the rapid increase of obesity in children. Owing to the deleterious effects of obesity and decreased physical activity on insulin sensitivity, the incidence of type 2 DM in U.S. children has increased by tenfold over the last generation. Several large multicenter studies have investigated the effects of lifestyle and/or differing pharmacologic agents on reducing the incidence of type 2 DM. In the Diabetes Prevention Program study (Diabetes Prevention Program Research Group, 2002), a lifestyle intervention consisting of 150 minutes of exercise per week and a 7% weight loss over 2.8 years reduced the incidence of type 2 DM by 58% compared with placebo. Metformin (1700 mg/day) reduced the progression by 31%. Interestingly, when metformin was stopped, its protective effect in preventing diabetes dissi-

pated rapidly. In the Tripod study, troglitazone (400 mg/day) for 30 months reduced the progression of type 2 DM by 55% in insulin-resistant high-risk Hispanic women (Buchanan *et al.*, 2002). This protective effect of troglitazone was maintained for at least 8 months after the drug was stopped. In the Stop-NIDDM study, acarbose (100 mg/three times a day) was given over a period of 3 years and produced a 25% reduction in the progression to type 2 DM (Chiasson *et al.*, 2002). *Orlistat*, a gastrointestinal lipase inhibitor used for weight loss, was administered over a 4-year period and resulted in a 37% reduction in the progression of type 2 DM in a group of insulin-resistant obese patients (Torgerson *et al.*, 2004). Finally, although the mechanisms are poorly understood, there are reports that angiotensin-converting enzyme inhibitors are associated with a decreased incidence of diabetes mellitus in high-risk patients (Scheen, 2004).

Based on the evidence that a variety of pharmacological agents can delay—and perhaps prevent—the onset of type 2 DM, multiple studies are underway investigating the effects of a range of pharmacologic agents in the prevention of type 2 DM.

Glucagon-like Peptide 1

Over four decades ago, McIntyre and colleagues reported that oral as compared with intravenous delivery of glucose produced a greater release of insulin. Subsequent work identified two hormones—*glucose-dependent insulinotropic polypeptide* (GIP) and *glucagon-like peptide* (GLP-1)—that are released from the upper and lower bowel that augment glucose-dependent insulin secretion. These hormones are termed *incretins*. The two incretins differentially stimulate insulin secretion. GIP has little effect on augmenting insulin secretion in type 2 DM, whereas GLP-1 significantly augments glucose-dependent insulin secretion. Consequently, GLP-1 has become an attractive target for therapeutic development in type 2 DM. GLP-1 also reduces glucagon secretion, slows gastric emptying, and decreases appetite. Thus, the compound may have unique properties to reduce postprandial glucose excursions (*i.e.*, increase in insulin, reduction of glucagon, slowing of gastric emptying) and also to induce weight loss. Offsetting these advantages, circulating GLP-1 is rapidly (1 to 2 minutes) inactivated by the dipeptidyl peptidase IV enzyme (DPP-IV). Thus, GLP-1 must be infused continuously to have therapeutic benefits. Consequently, considerable work has been performed to produce GLP-1 receptor agonists that maintain the physiologic effects of the native incretin but are resistant to the actions of DPP-IV. To date, two synthetic GLP-1 analogs have entered clinical trials. Exendin-4 is derived from the salivary gland of the Gila monster and has 53% homology with human GLP-1. Exendin-4 is resistant to DPP-IV and has full agonist activity at GLP-1 receptors. Several clinical studies have demonstrated that *exendin-4 (exenatide*, BYETTA) is effective in lowering hemoglobin A_{1c} (approximately 1% to 1.3%) and also promotes weight loss in type 2 DM. The compound is administered as twice-daily injections, although studies are planned to test a weekly or perhaps even a longer-acting formulation. Based on results of clinical trials, the FDA recently approved exenatide for twice-daily injection in combination therapy with other agents in subjects with type 2 DM. Reported exendin-4 side effects include a self-limiting nausea in 15% to 30% of patients; hypoglycemia can occur when GLP-1 agonists are used in conjunction with oral insulin secretagogues. A second long-acting analog of GLP-1, known as *NN2211*, is also in clinical trials. NN2211 contains a fatty acid moiety (hexadeconyl residue) covalently linked to GLP-1. NN2211 is resistant to the action of DPP-IV but also must be injected. Early clinical studies show that NN2211 is effective in lowering hemoglobin A_{1c} but may not induce as much weight loss as exendin-4. Nausea and hypoglycemia also occur with NN2211 when used with oral hypoglycemic agents.

An alternative approach to GLP-1 therapy is to inactivate the DPP-IV protease, thereby increasing endogenous circulating GLP-1 levels. A number of orally effective DPP-IV inhibitors have entered clinical trials. One study in type 2 DM reported similar reductions in hemoglobin A_{1c} as compared with the GLP-1 receptor analogs. These agents are well tolerated and appear to result in less nausea than the GLP-1 analogs. However, since DPP-IV can metabolize a wide range of peptides, there is a theoretical concern about the long-term safety of these compounds. Furthermore, the potency of the DPP-IV inhibitors may be limited by the amount of endogenous production of GLP-1. In contrast, pharmacological amounts of the injectable GLP-1 analogs can be administered with possibly increased therapeutic effect. Ongoing studies are currently being performed to further delineate the therapeutic effects of these agents, which offer promise for a novel pharmacotherapy in type 2 DM.

GLUCAGON

History. Distinct populations of cells were identified in the islets of Langerhans before the discovery of insulin. Glucagon was dis-

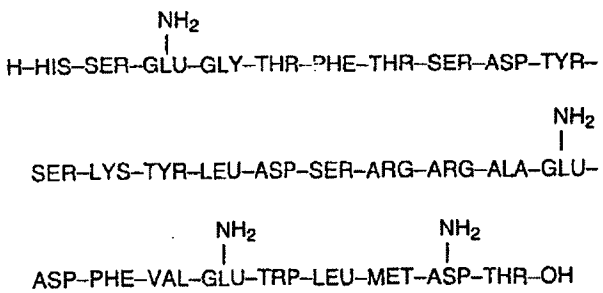


Figure 60–6. *The amino acid sequence of glucagon.*

covered by Murlin and Kimball in 1923, less than 2 years after the discovery of insulin. In contrast to the excitement caused by the discovery of insulin, few were interested in glucagon, and it was not recognized as an important hormone for more than 40 years. Glucagon has significant physiological roles in the regulation of glucose and ketone body metabolism but is only of minor therapeutic interest for the short-term management of hypoglycemia. It also is used in radiology for its inhibitory effects on intestinal smooth muscle.

Chemistry. Glucagon is a single-chain polypeptide of 29 amino acids (Figure 60–6). It has significant homology with several other polypeptide hormones, including secretin, vasoactive intestinal peptide, and gastrointestinal inhibitory polypeptide. The primary sequence of glucagon is identical in human beings, cattle, pigs, and rats.

Glucagon is synthesized from preproglucagon, a 180-amino-acid precursor with five separately processed domains. An amino-terminal signal peptide is followed by glicentin-related pancreatic peptide, glucagon, GLP-1, and glucagon-like peptide-2. Processing of the protein is sequential and occurs in a tissue-specific fashion; this results in different secretory peptides in pancreatic α cells and intestinal α-like cells (termed *L cells*). *Glicentin*, a major processing intermediate, consists of glicentin-related pancreatic polypeptide at the amino terminus and glucagon at the carboxyl terminus, with an Arg–Arg pair between. *Enteroglucagon* (or *oxyntomodulin*) consists of glucagon and a carboxyl-terminal hexapeptide linked by an Arg–Arg pair.

The highly controlled nature of the processing suggests that these peptides may have distinct biological functions. In the pancreatic α cell, the granule consists of a central core of glucagon surrounded by a halo of glicentin. Intestinal L cells contain only glicentin and presumably lack the enzyme required to process this precursor to glucagon. Enteroglucagon binds to hepatic glucagon receptors and stimulates adenylyl cyclase with 10% to 20% of the potency of glucagon. GLP-1 is an extremely potent potentiator of insulin secretion (*see* above), although it apparently lacks significant hepatic actions. Glicentin, enteroglucagon, and the glucagon-like peptides are found predominantly in the intestine, and their secretion continues after total pancreatectomy.

Regulation of Secretion. Glucagon secretion is regulated by dietary glucose, insulin, amino acids, and fatty acids. As in insulin secretion, glucose is a more effective inhibitor of glucagon secretion when taken orally than when administered intravenously, suggesting a possible role for gastrointestinal hormones in the response. The effect of glucose is lost in untreated or undertreated type 1 DM patients and in isolated pancreatic α cells, indicating that at least part of the effect is secondary to stimulation of insulin secretion. Somatostatin also inhibits glucagon secretion, as do free fatty acids and ketones.

Most amino acids stimulate the release of both glucagon and insulin. This coordinated response to amino acids may prevent insulin-induced hypoglycemia in individuals who ingest a meal of pure protein. Like glucose, amino acids are more potent when taken orally and thus may exert some of their effects *via* gastrointestinal hormones. Secretion of glucagon also is regulated by the autonomic innervation of the pancreatic islet. Stimulation of sympathetic nerves or administration of sympathomimetic amines increases glucagon secretion. *Acetylcholine* has a similar effect.

Glucagon in Diabetes Mellitus. Plasma concentrations of glucagon are elevated in poorly controlled diabetic patients. Because it enhances gluconeogenesis and glycogenolysis, glucagon exacerbates the hyperglycemia of diabetes. However, this abnormality of glucagon secretion appears to be secondary to the diabetic state and is corrected with improved control of the disease. The importance of the hyperglucagonemia in diabetes has been evaluated by administration of *somatostatin*. Although somatostatin does not restore glucose metabolism to normal, it significantly slows the rate of development of hyperglycemia and ketonemia in insulin-deficient subjects with type 1 DM. In normal individuals, glucagon secretion increases in response to hypoglycemia, but this important defense mechanism against insulin-induced hypoglycemia is lost in type 1 DM.

Degradation. Glucagon is degraded extensively in liver, kidney, plasma, and other sites of action. Its half-life in plasma is approximately 3 to 6 minutes. Proteolytic removal of the amino-terminal histidine residue leads to loss of biological activity.

Cellular and Physiological Actions. Glucagon interacts with a glycoprotein GPCR on the plasma membrane of target cells that signals through G_s (Mayo *et al.*, 2003) (*see* Chapter 1). The primary effects of glucagon on the liver are mediated by cyclic AMP. In general, modifications of the amino-terminal region of glucagon (*e.g.*, [Phe^1]glucagon and des-His$_1$-[Glu^9]glucagon amide) result in molecules that behave as partial agonists that retain some affinity for the glucagon receptor but have a markedly reduced capacity to stimulate adenylyl cyclase.

Glucagon activates phosphorylase, the rate-limiting enzyme in glycogenolysis, *via* cyclic AMP–stimulated phosphorylation, whereas concurrent phosphorylation of glycogen synthase inactivates the enzyme; glycogenolysis is enhanced, and glycogen synthesis is inhibited. Cyclic AMP also stimulates transcription of the gene for phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in gluconeogenesis. These effects normally are opposed by insulin, and insulin is dominant when maximal concentrations of both hormones are present.

Cyclic AMP also stimulates phosphorylation of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. This enzyme determines the cellular concentration of fructose-2,6-bisphosphate, which acts as a potent regulator of gluconeogenesis and glycogenolysis. When the concentration of glucagon is high relative to that of insulin, this enzyme is phosphorylated and acts as a phosphatase, reducing the concentration of fructose-2,6-bisphosphate in the liver. When the concentration of insulin is high relative to that of glucagon, the dephosphorylated enzyme acts as a kinase, raising fructose-2,6-bisphosphate concentrations. Fructose-2,6-bisphosphate interacts allosterically with phosphofructokinase-1, the rate-limiting

enzyme in glycolysis, increasing its activity. Thus, when glucagon concentrations are high, glycolysis is inhibited, and gluconeogenesis is stimulated. This also leads to a decrease in the concentration of malonyl CoA, stimulation of fatty acid oxidation, and production of ketone bodies. Conversely, when insulin concentrations are high, glycolysis is stimulated, and gluconeogenesis and ketogenesis are inhibited (*see* Foster, 1984).

Glucagon exerts effects on tissues other than liver, especially at higher concentrations. In adipose tissue, it stimulates adenylyl cyclase and increases lipolysis. In the heart, glucagon increases the force of contraction. Glucagon has relaxant effects on the gastrointestinal tract; this has been observed with analogs that apparently do not stimulate adenylyl cyclase. Some tissues (including liver) possess a second type of glucagon receptor that is linked to generation of IP_3, diacylglycerol, and Ca^{2+}. The role of this receptor in metabolic regulation remains uncertain.

Therapeutic Use. Glucagon is used to treat severe hypoglycemia, particularly in diabetic patients when intravenous glucose is not available; it also is used by radiologists for its inhibitory effects on the gastrointestinal tract.

All glucagon used clinically is extracted from bovine and porcine pancreas; its sequence is identical to that of the human hormone. For hypoglycemic reactions, 1 mg is administered intravenously, intramuscularly, or subcutaneously. The first two routes are preferred in an emergency. Clinical improvement is sought within 10 minutes to minimize the risk of neurological damage from hypoglycemia. The hyperglycemic action of glucagon is transient and may be inadequate if hepatic stores of glycogen are depleted. After the initial response to glucagon, patients should be given glucose or urged to eat to prevent recurrent hypoglycemia. Nausea and vomiting are the most frequent adverse effects.

Glucagon also is used to relax the intestinal tract to facilitate radiographic examination of the upper and lower gastrointestinal tract with barium and retrograde ileography and in magnetic resonance imaging of the gastrointestinal tract. Glucagon has been used to treat the spasm associated with acute diverticulitis and disorders of the biliary tract and sphincter of Oddi, as an adjunct in basket retrieval of biliary calculi, and for impaction of the esophagus and intussusception. Finally, it has been used diagnostically to distinguish obstructive from hepatocellular jaundice.

Glucagon releases catecholamines from pheochromocytomas and has been used experimentally as a diagnostic test for this disorder. Based on this effect, glucagon therapy is contraindicated in known pheochromocytoma. The hormone also has been used as a cardiac inotropic agent for the treatment of shock, particularly when prior administration of a β adrenergic receptor antagonist has rendered β adrenergic receptor agonists ineffective.

SOMATOSTATIN

Somatostatin was first isolated in 1973, following a search for hypothalamic factors that might regulate secretion of growth hormone from the pituitary gland (*see* Chapter 5). A potential physiological role for somatostatin in the islet was suggested by the observation that somatostatin inhibits secretion of insulin and glucagon. The peptide subsequently was identified in the δ cells of the pancreatic islet, in similar cells of the gastrointestinal tract, and in the central nervous system.

Somatostatin, the name originally given to a cyclic peptide containing 14 amino acids, is now known to be one of a group of related peptides. These include the original somatostatin (S-14), an extended 28-amino-acid peptide molecule (S-28), and a fragment containing the initial 12 amino acids of somatostatin-28 [S-28(1–12)]. S-14 is the predominant form in the brain, whereas S-28 is the main form in the gut. Acting *via* a family of GPCRs (*see* Chapter 55), somatostatin inhibits the release of thyroid-stimulating hormone and growth hormone from the pituitary gland; of gastrin, motilin, VIP, glicentin, and gastrointestinal polypeptide from the gut; and of insulin, glucagon, and pancreatic polypeptide from the pancreas.

Somatostatin secreted from the pancreas can regulate pituitary function, thereby acting as a true endocrine hormone. In the gut, however, somatostatin acts as a paracrine agent that influences the functions of adjacent cells. It also can act as an autocrine agent by inhibiting its own release in the pancreas. As the last cell to receive blood flow in the islets, the δ cell is downstream of the β and α cells. Thus, somatostatin may regulate the secretion of insulin and glucagon only *via* the systemic circulation.

Somatostatin is released in response to many of the nutrients and hormones that stimulate insulin secretion, including glucose, arginine, leucine, glucagon, VIP, and cholecystokinin. The physiological role of somatostatin has not been defined precisely. When administered in pharmacological doses, somatostatin inhibits virtually all endocrine and exocrine secretions of the pancreas, gut, and gallbladder. Somatostatin also can inhibit secretion of the salivary glands and, under some conditions, can block parathyroid, calcitonin, prolactin, and adrenocorticotropic hormone (ACTH) secretion. The α cell is about 50 times more sensitive to somatostatin than is the β cell, but inhibition of glucagon secretion is more transient. Somatostatin also inhibits nutrient absorption from the intestine, decreases intestinal motility, and reduces splanchnic blood flow.

Therapeutic uses of somatostatin are confined mainly to blocking hormone release in endocrine-secreting tumors, including insulinomas, glucagonomas, VIPomas, carcinoid tumors, and growth hormone–secreting adenomas (causing acromegaly). Because of its short half-life (3 to 6 minutes), substantial effort has been directed toward the production of longer-acting analogs. One such agent, *octreotide* (SANDOSTATIN), is available in the United States for treatment of carcinoid tumors, glucagonomas, VIPomas, and acromegaly. Another agent, *lanreotide*, is available in Europe. A depot form of octreotide administered intramuscularly every 4 weeks (SANDOSTATIN LAR) may be particularly suitable for chronic administration (*see* Chapter 55). Octreotide or lanreotide successfully controls excess secretion of growth hormone in most patients, and both have been reported to reduce the size of pituitary tumors in about one-third of cases. Octreotide also has been used to reduce the disabling form of diarrhea that occasionally occurs in diabetic autonomic neuropathy. Since octreotide also can decrease blood flow to the gastrointestinal tract, it has been used to treat bleeding esophageal varices, peptic ulcers, and postprandial orthostatic hypotension.

Gallbladder abnormalities (stones and biliary sludge) occur frequently with chronic use of the somatostatin analogs, as do gastrointestinal symptoms. Hypoglycemia, hyperglycemia, hypothyroidism, and goiter have been reported in patients being treated with octreotide for acromegaly.

DIAZOXIDE

Diazoxide is an antihypertensive, antidiuretic benzothiadiazine derivative with potent hyperglycemic actions when given orally (*see*

Chapter 32). Hyperglycemia results primarily from inhibition of insulin secretion. Diazoxide interacts with the ATP-sensitive K^+ channel on the β-cell membrane and either prevents its closing or prolongs the open time; this effect is opposite to that of the sulfonylureas. The drug does not inhibit insulin synthesis, and thus there is an accumulation of insulin within the β cell. Diazoxide also has a modest capacity to inhibit peripheral glucose utilization by muscle and to stimulate hepatic gluconeogenesis.

Diazoxide (PROGLYCEM) has been used to treat patients with various forms of hypoglycemia. The usual oral dose is 3 to 8 mg/kg per day in adults and 8 to 15 mg/kg per day in infants and neonates. The drug can cause nausea and vomiting and thus usually is given in divided doses with meals. Diazoxide circulates largely bound to plasma proteins and has a half-life of about 48 hours. Thus, the patient should be maintained at any dosage for several days before evaluating the therapeutic result.

Diazoxide has a number of adverse effects, including retention of Na^+ and fluid, hyperuricemia, hypertrichosis (especially in children), thrombocytopenia, and leucopenia, which sometimes limit its use. Despite these side effects, the drug may be quite useful in patients with inoperable insulinomas and in children with hyperinsulinism owing to nesidioblastosis.

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Review

Mexican plants with hypoglycaemic effect used in the treatment of diabetes

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Abstract

Diabetes mellitus is a syndrome which affects more and more people in all countries over the world. In México, it is commonly treated with herbal extracts. Such treatment may be of considerable benefit especially during the early stages of the illness. In this review, we discuss species commonly used in México in the treatment of diabetes. A total of 306 species have records of a popular use in the treatment of this syndrome in México. Seven of these species – *Cecropia obtusifolia* Bertol. (Cecropiaceae), *Equisetum myriochaetum* Schlecht & Cham (Equisetaceae), *Acosmium panamense* (Benth.) Yacolev (Fabaceae), *Cucurbita ficifolia* Bouché (Cucurbitaceae), *Agarista mexicana* (Hemsl.) Judd. (Ericaceae), *Brickellia veronicaefolia* (Kunth) A. Gray (Asteraceae), *Parmentiera aculeata* (*Kunth*) *Seem.* (Bignoniaceae) – are discussed in greater detail, highlighting our current knowledge about these botanicals, but also the enormous gaps in our knowledge, most notably as it relates to the species' toxicology, the pharmacokinetics of its active constituents and their metabolism.

Keywords: Type 2 diabetes; Hypoglycaemic plants; México; Neotropics; Traditional medicine

Contents

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1. Introduction

It is well known that diabetes mellitus is the commonest endocrine disorder that, according to the World Health Organization (WHO, 2004), affects more than 176 million people world wide, in México the WHO estimates that the number of diabetic patients will increase from more than 2 million in 2002 to more than 6 million in 2030, which would imply that in a few decades México may have highest rate of diabetes in the world. According to the Mexican health services, in 2001 diabetes was the first cause of mortality among the Mexican population (SSA, 2004). Because of the complications linked to diabetes like heart disease, retinopathy, kidney disease, and neuropathy, it also is a common cause of chronic morbidity and disability among the working population.

The term diabetes mellitus describes a metabolic disorder of multiple aetiologies and is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The causes of type 2 diabetes

are either insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect with or with out insulin resistance (WHO, 1999).

From an ethnopharmacological perspective, it is important to understand that this disease is one at the interface of conventional biomedical and local (or traditional) treatment. In México, limited data is available but based on our field experience diabetic patients practically always use plants with or without biomedical medication. Normally, patients are diagnosed in one of the primary health care centres and the MDs in these centres normally also prescribe appropriate medication. However, once a diagnosis is made the patients often recur to either local healers or to vendors of herbal and other health care products. Thus this is a disease for which many of the 'traditional' treatments were in fact developed in the last decades by local healers. In USA, some plant based compounds as well as herbal remedies are used along with other medications. In some cases, patients used these treatments instead of conventional medications, and severe complications including increased hospitalizations, ketoacidosis, and acute hyperglycaemia occurred (Shane-Mc Whorter, 2001). In Germany, at least two products for the treatment of diabetes, based on Mexican medicinal plants are available: Hando, Nopal (opuntia sp.), manufactured by Hando Austria and Sucontral (Coplachi: Hintonia sp.) manufactured by Harras Pharma, Munich.

There have been many studies on hypoglycaemic plants and a great variety of compounds have been isolated (alkaloids, glycosides, terpenes, flavonoids, etc.), but the main bottleneck is the further development of such 'leads' into clinically useful medicines and especially phytomedicines or adequate nutritional supplements, which would be of direct benefits to patients. In this context, it is important to remember that the modern drug metformin (a biguanide) is a derivate of an active natural product, galegine a guanidine isolated from the plant *Galega officinalis* L., which was used in the medieval times to relieve the intense urination in diabetic people (Witters, 2001).

In this review we summarize information on plants with current information in the international literature and highlight the current state of ethnopharmacological, phytochemical and clinical research on some of the more widely used and better known species.

2. An overview of important sources of information on Mexican antidiabetic plants

Several valuable reviews on the ethnobotanical use of plants of México are available (Martínez, 1954; Díaz, 1976; Aguilar et al., 1994; Argueta, 1994; Aguilar and Xolalapa, 2002). Other data can be found in many of the ethnobotanical thesis or monographs on specific regions. For México, we have documented at least 306 species from 235 genera and 93 families used as hypoglycaemic agents (see Table 1). The most commonly mentioned families are: Asteraceae (47 sp.), Fabaceae, (27), Cactaceae (16), Solanaceae and Euphorbiaceae (10) and Laminaceae (9).

But in our own experience from field work in Guerrero (Andrade-Cetto, 1995), when a directed ethnobotanical study is performed looking only from hypoglycaemic plants instead of a broad study looking for all medicinal plants, this number is at least double. Therefore, we estimate that there are about 500 species used by Mexican people to treat type 2 diabetes.

Starting at the early 1990s, important pharmacological studies were conducted by Alarcón Aguilar and Román-Ramos (Alarcón-Aguilar et al., 1997, 1998, 2000a, 2000b, 2002a, 2002b; Román-Ramos et al., 1991, 2001). In the beginning, this group tested several plants for their pharmacological activity in temporarily hyperglycaemic rabbits. Hyperglycaemia was induced with a glucose charge; later on they use healthy and alloxan-diabetic mice. Normally, the plant was process in the traditional way and the water extract tested. The authors have not looked for bioactive compounds of these species, with the exception of a detailed report on the pharmacology and phytochemistry of *Psacalium* sp.

The group of Pérez-Gutiérrez et al. (1996, 1998a, 1998c, 2000a, 2000b, 2001), Pérez-Gutiérrez and Vargas (2001) and Pérez et al. (1984, 1992) normally look for the pharmacological activity in normal and alloxan diabetic mice and rats. The group isolated bioactive compounds from some of the species working with the chloroform extracts.

The group of Andrade-Cetto (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001, 2004, and Wiedenfeld et al., 2000, 2003) generally starts with their own field studies. Then the traditionally used extract (normally water or butanol) is tested on Streptozotocin diabetic rats, from the active extract the main compounds are isolated and tested in the animal model.

There are some clinical studies like the well known one by Frati-Munari et al. (1983, 1987, 1989a, 1989b, 1989c, 1990, 1991a, 1991b), Castaneda-Andrade et al. (1997), Acosta-Patino (2001) (Revilla-Monsalve et al., 2002), and (Herrera-Arellano et al., 2004). Acosta Patiño and Revilla associated with the above described groups.

Finally we have recompilations in which the authors describe some aspects of species with hypoglycaemic effects from México (Pérez-Gutiérrez et al., 1998b) or some Mexican plants as part of world wide studies (Marles and Farnsworth, 1995; Ernst, 1997; Lamba et al., 2000). The detailed reviews of Shane-Mc Whorter (2001) and Yeh et al. (2003) on clinical aspects of anti-diabetic plants include, for example, the commonly used *Momordica charantia* L. (originally from Asia) and *Opuntia* sp. (a native of México). These relatively well known studied species are excluded from this review. Also, "Matarique" *Psacalium decompositum (Gray) H.E. Robins & Brett*, is not reviewed here. Research and development activities on this botanical lead to a patent on some compounds present in the plant with hypoglycaemic proprieties A detailed review was conducted as part of the efforts to obtain a patent (Inman et al., 1998): "the novel hypoglycemically active eremophilanolide sesquiterpenes which can be

Table 1
Main plants reported in México as Hypoglycemic, the original table is from Andrade, 1995, the data were actualized and 40 species added from Aguilar and Xolalpa 2002, the correct botanical names were corroborated at Missouri Botanical Garden (2004)—TROPICOS

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Abutilon lignosum (Cav.) D. Don	Sacxiu	Malvaceae	Root infusion		
Abutilon trisulcatum (Jacq.) Urban.	Tronadora	Malvaceae	Leaf boil		
Acacia retinodes Schltdl.	Mimosa	Fabaceae	Leaf boil		
Acourtia thurberi. (A. Gray) Reveal & R. M. King	Matarique	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	
Acrocomia mexicana Karw. ex Mart.	Coyol	Arecaceae	Root roasted, fruit raw	Alloxanic mice (+) Alloxanic mice (++) EtOH	Tetrahydropyrane, Coyolose
Agastache mexicana (Kunth) Lint et Epling	Toronjil	Lamiaceae	Plant (aerial) infusion		Essential oils
Agave atrovirens Karw. Ex Salm-Dyck	Maguey	Agavaceae	Steam macerated		Sapogeninns
Agave lecheguilla Torr.	Lechuguilla	Agavaceae	Steam macerated		
Agave salmiana Otto ex Salm-Dyck	Maguey	Agavaceae	Steam macerated		
Ageratina petiolaris Moc. & Sessé ex DC.	Hierba del ángel o Yolochichotl	Asteraceae	Plant (aerial) infusion		Terpens
Ageratum conyzoides L.	Hierba dulce	Asteraceae	Plant (aerial) infusion		Flavonoids, essential oils, terpens
Allionia choisyi Standl.	Hierba de la hormiga	Nyctaginaceae	Plant (aerial) infusion		
Allium cepa L.	Cebolla	Liliaceae	Bulbs raw		Sulfuric compounds
Alloispermum integrifolium (DC.) H. Rob.	Prodijiosa	Asteraceae	Plant (aerial) infusion		
Aloe barbadensis Mill.	Sábila	Liliaceae	Steam roasted, juice of the leaves	Normal rabbits (−)	Polysaccharides, flavonoids
Aloe vera (L.) Burm. F	Sábila	Liliaceae	Mixed with Nopal taken orally before meals	Normal mice (+)	Polysaccharides A B, flavonoids, terpens
Ambrosia artemisiifolia L.	Artemisa	Asteraceae	Plant (aerial) infusion		Sesquiterpen lactones
Anacardium occidentale L.	Marañon	Anacardiaceae	Bark infusion		
Ananas comosus (L.) Merr.	Piña	Bromeliaceae	Juice of the fruit		Monoterpenoids, Carotenoids, Lactones
Annona cherimola Mill.	Chirimoya	Annonaceae	Bark infusion		Isoquinolin Alkaloids
Annona glabra L.	Anona silvestre, palo de corcho	Annonaceae	Juice of the fruit root infusion		Diterpens, Alkaloids
Annona muricata L.	Guanabana	Annonaceae	Fruit raw		
Apodanthera buraeavi Cogn.	Pisto	Cucurbitaceae	Plant (aerial) infusion		
Aporocactus flagelliformis (L.) Lem.	Flor de junco	Cactaceae	Flowers infusion, steam infusion		
Arachis hypogaea L.	Cacahuate	Fabaceae	Seeds and oil		Sterols, flavonoids
Arceuthobium vaginatum (Humb. & Bonpl. ex Willd.) J. Presl	Injerto	Loranthaceae	Plant infusion		
Arctostaphylos pungens Kunth	Pingüica	Ericaceae	Leaves infusion, roots infusion		Tannins
Argemone mexicana L.	Chicalote, Cardo lechero.	Papaveraceae	Plant (aerial) infusion		Alkaloids, flavonoids
Argemone ochroleuca Sweet	Chicalote	Papaveraceae	Plant (aerial) infusion		Alkaloids
Argemone platyceras Link & Otto	Chicalote	Papaveraceae	Plant (aerial) infusion		
Aristolochia asclepiadifolia Brandegee	Guaco	Aristolochiaceae	Plant infusion EtOH		
Aristolochia malacophylla Standl.	Guaco	Aristolochiaceae	Flowers infusion		
Aristolochia sericea Benth.	Guaco	Aristolochiaceae	Steam infusion		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Artemisia absinthium L.	Ajenjo	Asteraceae	Leaf boil		Sesquiterpen lactones, flavonoids
Artemisia ludoviciana Nutt.	Estafiate	Asteraceae	Plant (aerial) infusion		
Artemisia vulgaris L.	Ajenjo	Asteraceae	Leaf boil		Sesquiterpens flavonoids
Asclepias linaria Cav.	Romerillo	Asclepiadaceae	Plant (aerial) infusion		Sterols, triterpenoids
Barosma betulina Bartl. & H.L. Wendl.	Buchū	Rutaceae	Leaves infusion		
Bauhinia divaricata L.	Pata de vaca	Fabaceae	Leaf boil, flowers boil	Normal rabbits (+)	
Begonia heracleifolia Schltdl. & Cham.	Mano de león	Begoniaceae	Steam infusion		
Berberis moranensis Schult. & Schult. f.	Palo muerto	Berberidaceae	Bark infusion		Cucurbitacines
Beta vulgaris L.	Betabel	Chenopodiceae	Juice of the leaves		Alkaloids, flavonoids
Bidens aurea (Aiton) Sherff	Té de milpa	Asteraceae	Plant (aerial) infusion		Essential oils
Bidens leucantha (L.) Willd.	Rosilla	Asteraceae	Plant (aerial) infusion	Alloxanic mice (++)	
Bidens odorata Cav.	Aceitilla, Mosote blanco	Asteraceae	Plant (aerial) infusion		Flavonoids, triterpens
Bidens pilosa L.	Aceitilla	Asteraceae	Plant (aerial) infusion	Alloxanic mice (+)	Flavonoids, triterpens
Bocconia arborea S. Watson	Llora sangre	Papaveraceae	Leaves infusion		Alkaloids
Puemus boldus Molina J. A. Schultes & J. H. Schultes in J. J. Roemer & J. A. Schultes	Boldo	Monimiaceae	Plant (aerial) infusion		
Bouvardia ternifolia (Cav.) Schltdl.	Trompetilla	Rubiaceae	Leaves, steam infusion		Bouvardin
Brickellia cavanillesii (Cass.) A. Gray	Prodigiosa	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	Essential oils, brikelin
Brickellia squarrosa B.L. Rob. & Seaton	Amula	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	Flavonoids
Brosimum alicastrum Sw.	Ojite	Moraceae	Bark infusion		Benzoquinones
Buchnera pusilla Kunth	Chichibé	Scrophulariaceae	Bark infusion		
Buddleia stachyoides Cham. & Schltd/.	Hierba del perro	Loganiaceae	Leaves infusion		Flavonoids, alkaloids, essential oils
Buddleja Americana L.	Tepozán	Loganiaceae	Leaves infusion		Flavonoids, alkaloids
Buddleja cordata Kunth	Tepozán	Loganiaceae	Leaves infusion		Alkaloids
Bursera simaruba (L.) Sarg.	Cuajiote	Burseraceae	Bark infusion		Tannins
Byrsonima crassifolia (L.) Kunth	Nanche	Malpighiaceae	Fruit, bark infusion		Triterpenoids
Cacalia decomposita A. Gray	Matarique	Asteraceae	Root infusion	Alloxan Mice (++)	Alkaloids, polysaccharides
Cacalia peltata Kunth	Matarique	Asteraceae	Root infusion	Normal rabbits (++)	Polysaccharides
Calamintha macrostema Benth.	Tabaquillo	Lamiaceae	Root infusion	Alloxanic mice (+)	
Calea hypoleuca B.L. Rob. & Greenm.	Prodigiosa	Asteraceae	Plant (aerial) infusion		
Calea integrifolia (DC.) Hemsl.	Prodigiosa	Asteraceae	Stem, infusion		Sesquiterpen lactones
Calea zacatechichi Schltdl.	Prodigiosa	Asteracae	Leaves infusion	Normal rabbits (+)	
Calliandra anomala (Kunth) J.F. Macbr.	Cabello de ángel	Fabaceae	Leaves infusion		Triterpenoid saponins
Callicarpa acuminata Kunth	Xpuk'im	Verbenaceae	Root, infusion		
Capraria biflora L.	Sabadilla	Scrophulariaceae	Leaves infusion	Alloxanic mice (+)	Alkaloids, loiflorin
Carica papaya L.	Papaya	Caricaceae	Latex		Monoterpenoids
Carya Nutt.	Nogal	Juglandaceae	Leaves infusion		
Casimiroa edulis La Llave & Lex.	Zapote blanco	Rutaceae	Leaves infusion, bark infusion		Alkaloids, casimiroin, edulein, edulinin
Cassia fistula L	Caña Fístula	Fabaceae	Fruit		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Cassia skinneri Benth.	Frijolillo	Fabaceae	Leaves infusion		
Cassia tomentosa L. f.	Retama cimarrona	Fabaceae	Leaves infusion		
Castela texana (T. & G.) Rose	Chaparro amargoso	Simaroubaceae	Bark infusion		Steroids
Castela tortuosa Liebm.	Venenilo	Simaroubaceae	Bark infusion		
Castilleja Mutis ex L. f.	Hierba del gato	Scrophulariaceae	Plant (aerial) infusion		
Catharanthus roseus (L.) G. Don	Vicaria	Apocinaceae	Root infusion		
Cecropia peltata L.	Guarambo	Cecropiaceae	Leaves infusion		
Ceiba pentandra (L.) Gaertn.	Ceiba, Pochote	Bombacaceae	Bark infusion		Essential oils
Centaurium brachycalyx Standl. & L.O. Williams	Tlanchalahua	Gentianaceae	Leaves infusion		
Centaurium calycosum (Buckley) Fernald	Tlanchalagua	Gentianaceae	Leaves infusion		
Chamaecrista hispidula (Vahl) H.S. Irwin & Barneby	Frijolillo	Fabaceae	Leaves infusion		
Chamaecrista hispidula (Vahl) H.S. Irwin & Barneby	Frijolillo	Fabaceae	Leaves infusion		
Chenopodium glaucum L.	Hierba del puerco	Chenopodiceae	Plant (aerial) infusion		
Chromolaena bigelovii (A. Gray) R.M. King & H. Rob	Ambula	Asteraceae	Plant (aerial) infusion		
Cirsium mexicanum DC.	Cardo santo	Asteraceae	Root infusion		
Cirsium rhaphilepis (Hemsl.) Petr.	Cardo santo	Asteraceae	Flower infusion		
Cissampelos pareira L.	Guaco	Menispermaceae	Root raw		Alkaloids, isoquinolin
Citrus aurantifolia (Christm.) Swingle	Limón	Rutaceae	Fruit		Essential oils, sesquiterpen lactones
Citrus limetta Risso	Lima	Rutaceae	Fruit		
Citrus sinensis (L.) Osbeck	Flor de azahar	Rutaceae	Ripe fruit infusion		Essential oils, flavonoids
Cnidoscolus aconitifolius (Mill.) I.M. Johnst.	Chaya	Euphorbiaceae	Leaves infusion		Polysaccharides
Cnidoscolus multilobus (Pax) I.M. Johnst.	Mala mujer	Euphorbiaceae	Leaves infusion		Triterpenoids, flavonoids, tannins
Cnidosculus chayamansa Mc Vaugh	Chayamansa	Euphorbiaceae	Leaves infusion		Flavonoids glycosides
Coix lacryma-jobi L.	Lágrima de San Pedro	Poaceae	Plant (aerial) infusion	Normal rabbits (+)	
Combretum farinosum Kunth	Bejuco de Carape	Combretaceae	Sap raw		
Conyza filaginoides (D C.) Hieron.	Simonillo	Asteraceae	Plant (aerial) infusion		Alkaloids, lenecin
Conyza gnaphalioides Kunth	Cimonillo, zacachichitl	Asteraceae	Leaves infusion		Terpens
Cordia elaeagnoides A. DC.	Cueramo	Boraginaceae	Bark infusion		Terpens
Cordia tinifolia Willd. Ex Roem. & Schult.	Palo mulato	Boraginaceae	Bark infusion		
Coriandrum sativum L.	Cilantro	Apiaceae	Plant (aerial) infusion		Coumarins, flavonoids, sesquiterpenoids, steroids
Costus mexicanus Liebm. ex Petersen	Caña de Jabalí	Zingiberaceae	Plant (aerial) infusion		
Costus rubber C. Wright ex Griseb.	Caña agria	Zingiberaceae	Plant (aerial) infusion		
Costus spicatus (Jacq.) Sw.	Caña de Jabalí	Zingiberaceae	Plant (aerial) infusion		
Crataegus mexicana Moc. & Sésse ex DC.	Tejocote	Rosaceae	Root infusion		
Crataegus pubescens (C. Presl) C. Presl	Tejocote	Rosaceae	Root infusion	Normal rabbits (++)	Tannins, flavonoids
Crotalaria acapulcensis Hook. & Arn.	Retama	Fabaceae	Leaves infusion		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Croton draco Schltdl.	Sangre de Grado	Euphorbiaceae	Cortex infusion, latex		Diterpens
Croton torreyanus Müll Arg.	Salvia	Euphorbiaceae			
Cucurbita maxima Duchesne	Calabaza	Cucurbitaceae	Fruit juice		Sterols, flavonoids
Cucurbita mexicana Damm	Calabaza, Melón	Cucurbitaceae	Leaves infusion fruit juice	Normal rabbits (++)	
Cuscuta jalapensis Schltdl.	Sacapal	Convolvulaceae	Steam infusion		
Cyathea fulva (M. Martens & Galeotti) Fée	Árbol de la vida	Cyatheaceae	Root infusion		
Cyathea fulva (Martens & Galeotti) Fée.	Árbol de la vida	Filicaceae	Leaves infusion		
Cynara scolymus L.	Alcachofa	Asteraceae	Fruit infusion, flowers infusion		Flavonoids, sesquiterpen lactones, fenolic acids
Cynodon dactylon (L.) Pers.	Grama	Poaceae	Plant (aerial) infusion	Normal rabbits (+)	Flavonoids, terpens
Daucus carota L.	Zanahoria	Apiaceae	Root juice		Cumarines, flavonoids, essential oils, fenolic acids
Diospyros digyna Jacq.	Zapote negro	Ebenaceae	Fruit		
Dorstenia contrajerva L.	Contrayerba	Moraceae	Leaves bolied		Alkaloids, cardenolids
Dyssodia micropoides (DC.) Loes.	Hierba pelotazo	Asteraceae	Plant (aerial) infusion		
Elaphoglossum sp. Schott ex J. Sm.	Hierba del pastor	Lomariopsidaceae	Plant (aerial) infusion		
Equisetum giganteum L.	Limpia plata	Equisetaceae	Plant (aerial) infusion		Flavonoids
Equisetum hyemale L.	Cola de caballo	Equisetaceae	Plant (aerial) infusion		Flavonoids, alkaloids
Eriobotrya japonica (Thunb.) Lindll.	Nispero	Rosaceae	Leaves infusion, flowers infusion	Normal rabbits (−)	Sesquiterpens, flavonoids
Eucalyptus globules Labill	Eucalipto	Myrtaceae	Leaves infusion	Alloxanic mice (+)	Flavonoids, terpens
Euphorbia maculata L.	Hierba de la Golondrina	Euphorbiaceae	Leaves infusion		
Euphorbia prostrata Aiton	Hierba de la Golondrina	Euphorbiaceae	Leaves infusion		Flavonoids
Eysenhardtia polystachya (Ortega) Sarg.	Palo dulce	Fabaceae	Plant (aerial) infusion, bark infusion	Alloxanic mice (+)	Flavonoids, triterpens
Foeniculum vulgare Mill.	Hinojo	Apiaceae	Plant (aerial) infusion		Essential oils, flavonoids
Fouquieria splendens Engelm.	Albarda	Fouquieriaceae	Leaves infusion		
Fraxinus alba Marshall	Fresno	Oleaceae	Leaves infusion bark infusion		
Gnaphalium oxyphyllum DC.	Gordolobo	Asteraceae			Diterpens, flavonoids
Guaiacum coulteri A. Gray	Guayacan	Zygophyllaceae	Bark infusion		Alkaloids
Guaiacum sanctum L	Guayacan	Zygophyilaceae	Bark infusion		
Guardiola angustifolia (A. Gray ex S. Watson) B.L. Rob.	Chintuza	Asteraceae			
Guardiola tulocarpus A. Gray	Chintuza	Asteraceae	Leaves infusion		
Guazuma ulmifolia Lam.	Guázima	Sterculiaceae	Bark infusion		Alkaloids, tannins
Haemahtoxylon brasiletto H. Karst.	Palo Brazil	Fabaceae	Bark infusion		
Hamelia patens Jacq.	Balletilla	Rubiaceae	Leaves infusion		Tannins
Haplopappus venetus (Kunth) S.F. Blake	Xapulli	Asteraceae	Plant (aerial) infusion		
Hechtia melanocarpa L. B. Sm.	Maguey agrio	Bromeliaceae	Steam raw		Flavonoids, alkaloids
Heterotheca inuloides Cass.	Arnica	Asteraceae	Leaves infusion		Flavonoids, essential oils
Hibiscus rosa-sinensis L.	Tulipán	Malvaceae	Plant (aerial) infusion		Sterols, flavonoids
Hidalgoa ternata La Llave	Mozote de monte	Asteraceae	Plant (aerial) infusion		
Hintonia latiflora (Sesse & Moc. ex DC.) Bullock	Copalquin, Cáscara sagrada.	Rubiaceae	Bark infusion	Alloxanic mice (++)	Neoflavonoid, coutareagenin.

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Hippocratea excelsa Kunth	Cancerina	Hippocrateaceae	Root infusion		Sesquiterpens
Ipomoea starts Cav.	Tumba vaquero	Convolvulaceae	Plant (aerial) infusion		Essential oils
Jatropha dioica Cerv.	Sangre de grado	Euphorbiaceae	Root infusion		
Jatropha elbae J. Jiménez Ram.	Sangre de grado	Euphorbiaceae	Bark infusion		Terpens, flavonoids
Juliania adstringens (Schltdl.) Schltdl.	Cuachalalate	Julianiaceae	Bark infusion		Triterpens
Justicia spicigera Scheltdl	Muicle	Acanthaceae	Leaves infusion		Flavonoids
Kalanchoe pinnata (Lam.) Pers.	Tronador	Crassulaceae	Plant (aerial) infusion		Flavonoids
Karwinskia humboldtiana (Willd. ex Roem. & Schult.) Zucc.	Tullidora	Rhamnaceae	Leaves infusion		
Kohleria sp. Regel	Tlanchichinoli	Gesneriaceae	Leaves infusion		Triterpens
Larrea tridentata (Sessé & Moc. ex DC.) Coville	Gobernadora	Zygophylaceae	Plant (aerial) infusion		Terpens, lignans
Lepechinia caulescens (Ortega) Epling	Bretónica	Lamiaceae	Leaves infusion	Alloxanic mice (++)	Terpens
Lepidium virginicum L.	Lentejilla	Brassicaeae	Leaves infusion		
Leucaena leucocephala (Lam.) de Wit	Guaje	Fabaceae	Seed raw		Tannins
Leucophyllum texanum Benth.	Cenicillo	Scrophulariaceae	Plant (aerial) infusion		
Ligusticum porteri J.M. Coult. & Rose	Raiz de cochino	Apiaceae	Root infusion		Essential oils
Ligustrum japonicum Thunb.	Fresno	Oleaceae	Leaves infusion		
Loeselia coccinea (Cav.) G. Don	Hoja de la virgen	Polemoniaceae	Leaves infusion		Alkaloids, saponins
Loeselia mexicana (Lam.) Brand	Hierba de la virgen.	Polemoniaceae	Leaves infusion	Alloxanic mice (+)	Alkaloids, essential oils
Lonchocarpus cruentus Lundell	Guayacán	Fabaceae	Bark infusion		
Lopezia racemosa Cav.	Perilla	Onagraceae	Plant (aerial) infusion		
Lophocereus schottii (Engelm.) Britton & Rose	Muso	Cactaceae	Steam infusion		Alkaloids
Lysiloma acapulcense (Kunth.) Benth	Tepehuaje	Fabaceae	Leaves infusion, bark infusion		Tannins
Malmea depresa (Baillon) Fries	Elemuy	Anonaceae	Root infusion		Flavonoids
Malvastrum coromandelianum (L.) Garcke	Marvavisco	Malvaceae	Leaves infusion		Tannins
Mangifera indica L.	Mango	Anacardiaceae	Bark infusion leaves infusion		Flavonoids, essential oils, terpens
Marrubium vulgare L.	Marrubio	Lamiaceae	Leaves infusion, root infusion	Normal rabbits (++)	Terpens, flavonoids
Melothria pendula L.	Sandiita	Cucurbitaceae	Plant (aerial) infusion		
Mentha piperita L.	Hierbabuena	Lamiaceae	Leaves infusion		Essential oils, terpens, flavonoids
Mentha rotundifolia (L.) Huds.	Mostranza	Lamniaceae	Leaves infusion		Essential oils, terpens
Mentha suaveolens Ehrh.	Mastranzo	Lamiaceae	Leaves infusion		
Mimosa zygophylla Benth.	Gatuño	Fabaceae	Leaves infusion		
Mirabilis jalapa L.	Maravilla	Nyctaginaceae	Plant (aerial) infusion		Triterpens, flavonoids
Momordica charantia L.	Cundeamor,	Cucurbitaceae	Leaves infusion		Terpens, steroids, flavonoids
Morus nigra L.	Moral negro	Moraceae	Leaves infusion		
Musa sapientum L.	Flor de plátano	Musaceae	Root infusion		
Nasturtium officinale R. Br.	Berro	Brassicaeae	Plant (aerial) infusion		Flavonoids, alkaloids, terpens
Nopalea cochenillifera (L.) Salm-Dyck	Nopal	Cactaceae	Steam raw		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Nopalea inaperta Schott ex Griffiths.	Nopal	Cactaceae	Steam raw		
Olea europaea L.	Hierba de oliva	Oleaceae	Leaves infusion		Alkaloids, flavonoids, terpens
Opuntia atropes Rose	Nopal bianco	Cactaceae	Steam raw		
Opuntia ficus-indica (L.) Mill.	Nopal	Cactaceae	Steam raw		Alkaloids, flavonoids
Opuntia fulgida Engelm.	Choya	Cactaceae	Steam raw		
Opuntia guilanchi Griffiths	Nopal blanco	Cactaceae	Steam raw		
Opuntia imbricata (Haw) DC.	Xoconostle	Cactaceae	Steam raw, fruit		
Opuntia leucotricha DC.	Duraznillo	Cactaceae	Steam		
Opuntia megacantha Salm-Dyck	Nopal blanco	Cactaceae	Steam raw		
Opuntia streptacantha Lem	Nopal	Cactaceae	Steam raw	Normal rabbits (+)	
Pachira aquatica Aubl.	Zapote de agua	Bombacaceae	Bark infusion		
Pachycereus marginatus (DC.) Britton & Rose	Organo, Sahuaro	Cactaceae	Steam raw		
Pachycereus pringlei (S. Watson) Britton & Rose	Cardón	Cactaceae	Steam raw		
Packera candidissima (Greene) W.A. Weber & Á. Löve	Lechugilla	Asteraceae	Plant (aerial) infusion		
Parathesis lenticellata Lundell	Chagalapoli	Myrsinaceae	Leaves infusion		
Parkinsonia aculeata L.	Bagote	Fabaceae	Leaves infusion		Flavonoids, triterpens
Parthenium hysterophorus L	Escobilla	Asteraceae	Plant (aerial) infusion		Alkaloids, partenin
Pavonia schiedeana Steud	Cadillo	Malvaceae	Leaves infusion	Normal rabbits (−)	Tannins
Persea americana Mill	Aguacate	Lauraceae	Leaves infusion		Sterols, flavonoids
Petroselinum crispum (Mill.) Nyman ex A.W. Hill	Perejil	Apiaceae	Plant (aerial) infusion		Essential oils, flavonoids
Phaseolus vulgaris L.	Fríjol	Fabaceae	Fruit infusion	Normal rabbits (+)	Essential oils, flavonoids, alkaloids
Phlebodium aureum (L.) J. Sm.	Calahuala	Polypodiaceae	Root infusion		Steroids
Phoradendron bolleanum (Seem.) Eichler	Injerto	Viscaceae	Plant (aerial) infusion		
Phoradendron tomentosum (DC.) Engelm. ex A. Gray	Muicle	Viscaceae	Plant (aerial) infusion		Phoratoxins
Phragmites australis (Cav.) Trin. ex Steud.	Carrizo	Poaceae	Plant (aerial) infusion		
Physalis coztomatl Dunal	Costomate	Solanaceae	Leaves infusion		
Physalis philadelphica Lam.	Tomate	Solanaceae	Fruit roasted	Normal rabbits (−)	
Piper auritum Kunth	Acoyo	Piperaceae	Leaves infusion		Terpens, flavonoids, essential oils
Piper hispidum Sw.	Cordoncillo	Piperaceae	Leaves infusion		
Piper sanctum (Miq.) Schltdl. ex C. DC.	Hierba Santa	Piperaceae	Leaves infusion		Essential oils, alkaloids
Piper schiedeanum Steud.	Tlaxalisnuat	Piperaceae	Leaves infusion		
Pithecellobium dulce (Roxb.) Benth.	Guamúchil	Fabaceae	Bark infusion		
Plantago australis Lam.	Gusanillo	Plantaginaceae	Plant (aerial) infusion		Lignans
Plantago major L.	Llante	Plantaginaceae	Plant infusion		Flavonoids, terpens
Plumbago scandens L.	Plumbago	Plumbaginaceae	w/i		Flavonoids
Plumeria rubra L.	Flor de mayo	Apocynaceae	Flowers infusion		
Polygonum acre Lam.	Sanguinaria	Polygonaceae	Leaves infusion		
Populus alba L.	Abedúl	Salicaceae	Leaves infusion		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Porophyllum punctatum (Mill.) S.F. Blake	Piojillo	Asteraceae	Flowers infusion		
Portulaca denudata Poelln.	Verdolaga	Portulacaceae	Plant (aerial) infusion		
Portulaca oleracea L.	Verdolaga	Portulacaceae	Plant (aerial) infusion		Alkaloids, terpens
Pouteria hypoglauca (Standl.) Baehni	Baehni	Sapotaceae	Leaves infusion		
Prosopis juliflora (Sw.) DC.	Mezquite	Fabaceae	Fruit raw		
Prunus serotina subsp. capuli (Cav.) McVaugh	Capulin	Rosaceae	Fruit infusion		Terpens
Psacalium sinuatum (Cerv.) H. Rob. & Brettell	Matarique	Asteraceae	Root infusion		
Pseudosmodingium pemiciosum (Kunth) Engl.	Cuajilote	Anacardiaceae	Root infusion bark infusion		
Psidium guajava L.	Guayaba	Myrtaceae	Fruit		Terpens, flavonoids
Psidium yucatanense Lundell	Pach	Myrtaceae	Bark infusion		
Psittacanthus calyculatus (DC.) G. Don	Muérdago	Loranthaceae	Plant infusion, flowers infusion	Alloxanic mice (++)	
Quassia amara L.	Cuasia	Simaroubaceae	Leaves infusion		Alkaloids, terpens
Quercus acutifolia Neé	Encino	Fagaceae	Bark infusion		Terpens, flavonoids
Quercus rugosa Neé	Encino	Fagaceae	Bark infusion		
Randia echinocarpa Moc. & Sessé ex DC.	Grangel	Grangel	Leaves infusion		
Randia echinocarpa Moc. & Sessé ex DC.	Granjil	Rubiaceae	Fruit		
Raphanus sativus L.	Rábano	Brassicaceae	Root infusion		
Rhipsalis baccifera (J.S. Muell.) Steam	Niguilla	Cactaceae	Stem infusion, fruit raw		
Rhizophora mangle L.	Mangle	Rhizophoraceae	Bark infusion		Tannins
Ricinus communis L.	Huiguerilla	Euphorbiaceae	Leaves infusion		Flavonoids, terpens
Rosa centifolia L.	Rosa de castilla	Rosaceae	Leaves infusion		
Rubus adenotrichus Schltdl.	Zarzamora	Rosaceae	Leaves infusion		
Russelia equisetiformis Schltdl. & Cham.	Cola de caballo	Scrophulariaceae	Plant (aerial) infusion		
Salix taxifolia Kunth	Taray	Salicaceae	Steam infusion		
Salpianthus arenarius Humb. & Bonpl.	Catarinita	Nyctaginaceae.	Leaves infusion	Normal rabbits (++)	
Salvia leucantha Cav.	Salvia morada	Lamiaceae	Plant (aerial) infusion		Terpens
Samvitalia procumbens Lam.	Ojo de gallo	Asteraceae	Plant (aerial) infusion		Terpens
Saurauia pringlei Rose	Picon	Actnidaceae	Leaves infusion		
Sechium edule (Jacq.) Sw.	Chayote	Cucurbitaceae	Fruit raw		Flavonoids
Sedum dendroideum Moc. & Sessé ex DC.	Siempreviva	Crassulaceae	Plant (aerial) infusion		Sedoheoptulose
Sedum moranense HBK.	Siempreviva	Crassulaceae	Plant (aerial) infusion		
Sedum praealtum A. DC.	Siempreviva	Crassulaceae	Leaves infusion		
Selaginella lepidophylla (Hook. & Grev.) *Spring*	Doradilla	Selaginellaceae	Plant (aerial) infusion		Essential oils
Selaginella pallescens (C. Presl) Spring	Flor de piedra	Sellaginacae	Plant (aerial) infusion		
Selloa plantaginea Kunth	Diente de elef.ante	Asteraceae	Plant (aerial) infusion		
Senecio albo-lutescens Sch. Bip.	Matarique	Asteraceae	Root infusion		
Senecio palmeri A. Gray	Matarique	Asteraceae	Root infusion		
Senecio peltiferus Hemsl.	Matarique	Asteraceae	Root infusion		
Senna multiglandulosa (Jacq.) H.S. Irwin & Barneby	Retama china	Fabaceae	Leaves infusion		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Senna obtusifolia L. (L.) H.S. Irwin & Barneby	Pa xojk	Fabaceae	Leaves infusion		Antraquinones, emodin
Senna occidentalis (L.) Link	Frijolillo	Fabaceae	Root infusion		Flavonoids, sterols
Serjania racemosa Schumach.	Bejuco tres en uno.	Sapindaceae	Plant (aerial) infusion		
Serjania triquetra Radlk.	Bejuco de tres C.	Salicaceae	Bark infusion		
Simira sp. Aubl	Quina roja, cáscara sagrada	Rubiaceae	Bark infusion		
Smilax aristolochiifolia Mill.	Zarzaparrilla	Similicaceae	Root infusion		Sapogenins
Solandra nitida Zuccagni	Flor de guayacán	Solanaceae	Flower infusion		
Solanum americanum Mill.	Hierba mora	Solanaceae	Plant (aerial) infusion		Alkaloids, solanin
Solanum brevistylum Wittm	Malabar	Solanaceae	Plant (aerial) infusion		
Solanum diversifolium Dunal	Malabar	Solanaceae	Leaves infusion	Normal rabbit (++)	
Solanum nigrescens M. Martens & Galeotti	Hierba mora	Solanaceae	Plant (aerial) infusion		
Solanum rostratum Dunal	Duraznillo	Solanaceae	Plant (aerial) infusion		
Solanum torvum Sw.	Berenjena	Solanaceae	Root infusion		
Solanum verbascifolium C.B. Wright	Berenjena	Solaneceae	Plant (aerial) infusion		Steroidal, alkaloids
Sonchus oleraceus L.	Lechuguilla	Asteraceae	Leaves infusion		Flavonoids
Spartium junceum L.	Retama	Fabaceae	Leaves infusion		
Sphaeralcea angustifolia (Cav.) G. Don	Hierba del negro	Malvaceae	Plant (aerial) infusion		
Stachytarpheta jamaicensis (L.) *Vahl*	Verbena	Verbenaceae	Plant (aerial) infusion		Terpens
Stenocereus marginatus (DC.) Berger & Buxb	Organo de Zopilote	Cactaceae	Steam roasted		
Struthanthus densiflorus (Benth.) Standl.	Injerto	Loranthaceae	Leaves infusion		
Swietenia humilis Zucc.	Zopilote	Meliaceae.	Seed raw		
Tagetes erecta L.	Cempasuchil o Flor de muerto,	Asteraceae	Plant (aerial) infusion		Terpens, essential oils
Tamarindus indica L.	Tamarindo	Fabaceae	Pulp of fruit raw		Flavonoids
Taraxacum officinale Weber ex F.H. Wigg.	Diente de león	Asteraceae	Leaves infusion		Terpens
Taxodium mucronatum Ten.	Ahuehuete	Taxodiaceae	Leaves infusion		Flavonoids
Tecoma stans (L.) Juss. ex Kunth	Tronadora	Bignoniaceae	Leaves infusion, plant infusion plant infusion	Alloxanic mice (++) Normal Dogs (++)	Alkaloids, terpens
Terminalia catappa L.	Castaño	Combretaceae	Fuit		
Teucrium cubense Jacq.	Agrimonia	Lamiaceae	Leaves infusion	Normal rabbits (+)	
Thriallis glauca (Cav.) Kuntze	Amargoso	Malphigiaceae	Root infusion		Flavonoids, terpens
Tillandsia usneoides (L.) L.	Heno	Bromeliaceae	Plant (aerial) infusion	Alloxanic mice (++)	Flavonoids
Tournefortia hirsutissima L.	Lagrima de San Pedro.	Boraginaceae	Steam infusion		
Tournefortia petiolaris DC.	Lagrima de San Pedro.	Boraginaceae	Steam infusion		
Tradescantia pendula (*Schnizl.*) D.R. Hunt	Comellina	Commelinaceae	Leaves infusion		Flavonoids
Trigonella foenum-graecum L.	Fenogreco	Fabaceae	w/i		
Tropaeolum majus L.	Mastuerzo	Tropaeoleaceae	Leaves infusion		
Turnera diffusa Willd ex Schult.	Damiana.	Turneraceae	Leaves infusion		Flavonoids, terpens
Urtica dioica L.	Ortiga	Urticaceae	Plant (aerial) infusion	Normal rabbits (−)	Flavonoids, coumarins
Urtica mexicana Liebm.	Ortiga	Urticaceae	Leaves infusion		

Table 1 (*Continued*)

Scientific name	Common name	Family	Plant part used and preparation	Pharmacological STudies*	Phytochemical informat**
Valeriana edulis Nutt. ex Torr. & A. Gray	Valeriana	Valerianaceae	Root infusion		
Valeriana procera Kunth	Valeriana	Valerianaceae	Root infusion	Alloxanic mice (−)	
Verbesina crocata (Cav.) Less.	Capitaneja	Asteraceae	Leaves infusion	Alloxanic mice (+)	
Verbesina persicifolia DC.	Huichin	Asteraceae	Plant (aerial) infusion	Alloxanic mice (+)	Sesquiterpens
Zaluzania angusta (Lag.) Sch. Bip.	Limpia tuna	Asteraceae	Root infusion		
Zantoxylum fagara L.	Tankaschè	Rutaceae	Leaves infusion		Alkaloids
Zea mays h.	Pelos de elote	Poaceae	Fruit infusion		
Zexmenia gnaphalioides A. Gray	Peonia	Asteraceae	Root infusion		
Zizyphus acuminate Benth	Corongoro, amol	Rhamnaceae	Plant (aerial) infusion		

* In the Animal studies +, indicates activity and the level of it, while—mean no observed activity for the tested extract.
** The phytochemical information, refers about the reports for the plant no the active compounds.

isolated from *Psacalium* spp., processes for obtaining the novel eremophilanolide sesquiterpenes and methods for their use as hypoglycemic agents, for example, in the treatment of diabetes." Sadly, Mexicans have had no say in developing this patent on a Mexican plant.

Instead we review the current information of some lesser known plants commonly used in México to treat type 2 diabetes and summarise and discuss ethnobotanical, pharmacognostical, phytochemical, pharmacological and clinical data for the main species reported as hypoglycaemic in México (Table 1).

3. Ethnopharmacology of commonly used antidiabetic plants in México

Seven species used throughout México, reported in the international literature with pharmacological and phytochemical studies are discussed in greater detail and their potential for developing phytomedicines with a validated profile of activity and demonstrated safety profile is analysed (Table 2).

3.1. Cecropia obtusifolia Bertol. (Cecropiaceae)

The hypoglycaemic effect of this plant sold on several markets as a treatment for type 2 diabetes is well known in México, DF (Andrade-Cetto, 1999) and it is also known from many ethnobotanical collections in rural lowland areas (e.g. Heinrich, 1989).

3.1.1. Botanical description

A monopodic tree 20 m tall, growing in secondary vegetation in the tropical rain forest. This tree has a tall, straight, hollow trunk and a stratified treetop with few large branches growing horizontally from the trunk. The leaves are in a spiral disposition located at the top of the branches and are simple, peltate or deeply palmate, with a deep green colour in the upper face and grey at the lower surface. It is a fast-growing pioneer tree from tropical America, the hollow septate twigs are inhabited by ants (Pennington and Sarukhán, 1998).

3.1.2. Distribution

It is widespread in México, along both coasts, from Tamaulipas and San Luis Potosi to Tabasco on the Gulf of México, and from Sinaloa to Chiapas on the Pacific side. It is, in fact, a weedy species, which would presumably be relatively easy to grow on a larger scale or to harvest it sustainably by collecting material in the first few years after a *milpa* (corn field) has been given up.

3.1.3. Ethnobotany

Traditionally the dry leaves (15 g) are boiled in water (500 ml), the resulting infusion is cooled in the pot, then filtrated and drunk as "agua de uso". The cold infusion is consumed over the day or when the people have thirst. The use is reported from the following Mexican states, Hidalgo, Guerrero, Veracruz, Yucatan, Campeche, Tabasco, Edo. de México, Oaxaca and Chiapas. The traditional names include "Guarumbo", "Chancarro", "Hormiguillo", "Chiflon" and "Koochlé" among others.

3.1.4. Main constituents

The following constituents have been reported: β-sitosterol, stigmasterol, 4-ethyl-5-(*n*-3valeroil)-6-hexahydrocoumarin and 1-(2-methyl-1-nonen-8-il)-aziridine (Argueta, 1994). The type of extract for the isolated compounds has not been specified. From the butanolic extract Andrade-Cetto and Wiedenfeld (2001) isolated chlorogenic acid and isoorientin (Fig. 1 compounds **1** and **2**). The isolated compounds are also found in the medicinal tea.

3.1.5. Pharmacology

A hypoglycaemic effect of the water extract was demonstrated in alloxan diabetic mice (Pérez et al., 1984), in hyperglycaemic rabbits (Román-Ramos et al., 1991) and in

Table 2
Overview on antidiabetic effects of the seven species reviewed and commonly used in México (for references see text)

Botanical species	*Cecropia obtusifolia*	*Equisetum myriochaetum*	*Acosmium panamense*	*Cucurbita ficifolia*	*Agarista mexicana*	*Brickellia veronicaefolia*	*Parmentiera aculeata*
Animal model	Streptozotocin diabetic rats.	Streptozotocin diabetic rats	Streptozotocin diabetic rats	Hyperglycaemic rabbits Alloxan mice and rats	Normal and alloxan mice and rats	Normal and alloxan mice	Normal and alloxan mice
Clinical studies	In progress	In humans, positive	None	In humans, positive	None	None	None
Bioactive compounds	Chlorogenic acid (1), isoorientin (2)	Kaempferol-3-*O*-sophoroside, kaempferol-3,7-di-*O*-β-glucoside, caffeoyl-methylate-4-β-glucopuranoside kaempferol-3-*O*-sophoroside-4′-*O*-β-glucoside (3)	Caffeic acid desmethyl yangonine its O$^{4'}$-mono and di(1–6) glucoside (4 and 5)	Unknown	12-ursene 23,24 dimethyl 1-24-ethyl-sigmast-25-ene (6 and 7)	5,7,3′-trihydroxy-3,6,4′-trimethoxy flavone (8)	Lactucin-8-*O*-methylacrylate

Streptozotocin diabetic rats (Andrade-Cetto et al., 2000). Pérez-Guerrero et al. (2001) performed several pharmacological tests in male Swiss albino mice and concluded that the water extract of the leaves has low toxicity, a substantial effect as a central depressor, anti-inflammatory and analgesic effects. The report of Pérez et al. (1984) shows activity after 5 h of intraperitonal and oral administration of the aqueous extract (obtained from 50 g leaves boiled in 250 ml distilled water). This study does not give more details about the effects between time 0 and 5 h. Also, a proper positive control like glibenclamide is missing. There is no statistical evaluation of the data and the dose administered to each animal is not mentioned. The study by Román-Ramos et al. (1991) does not use a proper diabetic animal model (Versphol, 2002). Instead, it was conducted in healthy rabbits obtaining a glucose tolerance curve. The effect of the aqueous extract of 132 g leaves boiled in 1 l water and administering the infusion (4 ml/kg) using a gastric tube showed a significant hypoglycaemic effect at 60 min after the administration of the extract, and showed no activity after 4 and 5 h. Since the amount of dry extract administered was not measured in either study, the actual doses are missing. Also, in the study performed by Pérez et al. the reported activity is after 300 min (5 h) and Roman-Ramos et al. report no activity at this time.

In the study by Andrade-Cetto and Wiedenfeld (2001) in Streptozotocin diabetic rats, a positive and an untreated control was used, the water and butanolic extracts as well as the isolated compounds were tested, the hypoglycaemic effect is observed from 60 to 180 min for all the tested samples, with statistical significance. However, according to Versphol (2002) the animal model resembles more type I diabetes than type 2, while Islas-Andrade et al. (2000) provided evidence that using this model in a proper way diabetes type 2 can be mimicked.

Herrera-Arellano et al. (2004) performed a study on diabetic type 2 people, they conclude that the plant has a significant hypoglicemic effect after 21 days of oral administration, of 3 g/day of the plant. The Cecropia group was also treated with glibenclamide at different doses, and no proper controls were used, so there is no point of comparison, and the effect can not be only attributed to the extract. The authors argue that the plant was given in a similar way as the traditional preparation, but the traditional preparation takes between 12 and 15 g plant/day.

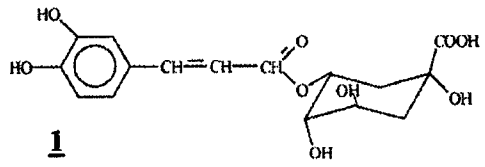
3.1.6. Possible mechanism of action

Chlorogenic acid was identified as a specific inhibitor of the glucose-6-phosphate translocase component (Gl-6-P translocase) in microsomes of rat liver (Hemmerle et al., 1997). Simultaneous targeting of gluconeogenesis and glycogenolysis with an inhibitor of Gl-6-P translocase would result in a reduction of hepatic glucose production. The action of chlorogenic acid may well explain the hypoglycaemic effect observed by Pérez et al. (1984). The hypoglycaemic effect observed in mice after 5 h of experiment may be due to a lack of hepatic glucose production resulting in a hypogly-

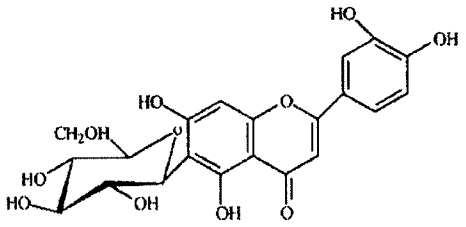
caemic state. This would have been caused by the liver not providing glucose due to the action of cholorogenic acid during the fasting of the animals. In the work by Román-Ramos et al. (1991), the animals were not fasted and they received an oral glucose charge at times 0 and 60 min at a dose of 2 g/kg. The authors did not observe any hypoglycaemic effect after 5 h, they argued that with this animal model glycaemia reaches basic values within 300 min. If the basic glycaemic value is reached at 300 min then the hepatic production of glucose has not been trigger, and there was no hypoglycaemic effect observed, and of course, no action of the cholorogenic acid.

The other compound isolated by Andrade-Cetto and Wiedenfeld (2001), isoorientin, had previously been tested by Afifi et al. (1999). They showed that the compound caused concentration-dependent inhibition of the amplitude and frequency of the phasic contractions of the rat and guinea-pig uterus but did not affect the isolated aorta, ileum or trachea. Deliorman-Orhan et al. (2003) tested the hepatoprotective activity of *Gentiana olivieri* and conclude that the effect "might possibly [be] due to the potent antioxidant activity of isoorientin". The antioxidant effect of plants used in diabetes treatment was shown by Letitia et al., 2002. According to these authors, the benefits of antioxidants in the prevention of the complications of diabetes supports and validates the use of the traditional medicine. Antioxidants are important in preventing diabetes, with low levels of plasma antioxidants implicated as a risk factor for the development of the disease,

Cecropia obtusifolia

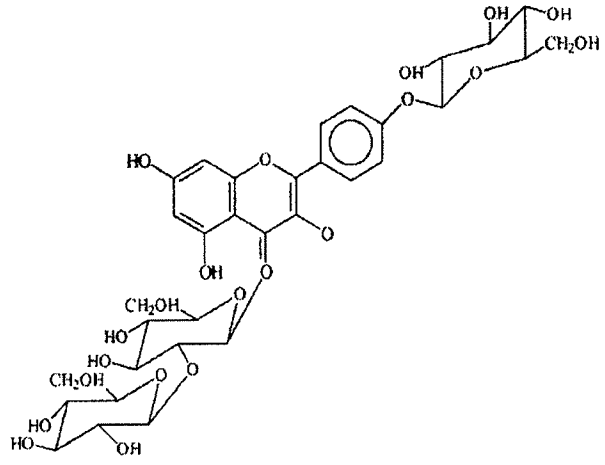


1



2

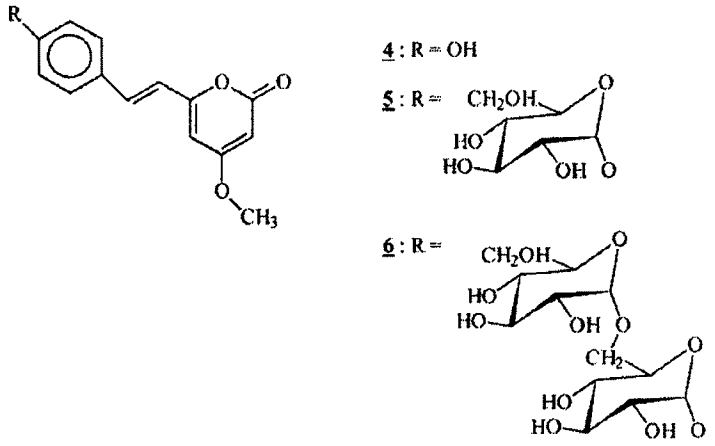
Equisetum myriochaetum



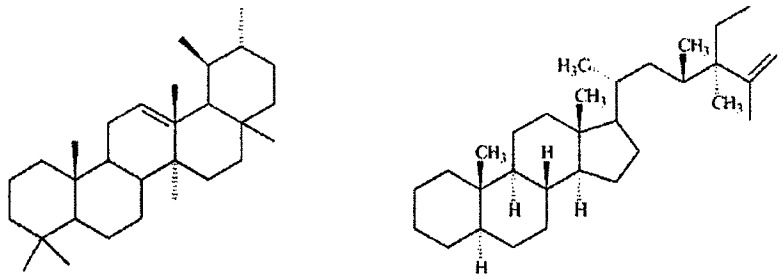
3

Fig. 1. Natural products with documented hypoglycaemic effects from the species discussed in detail in this review.

Acosmium panamense

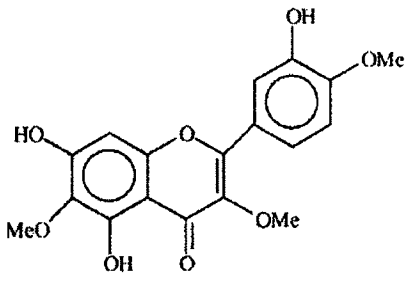


Agarista mexicana

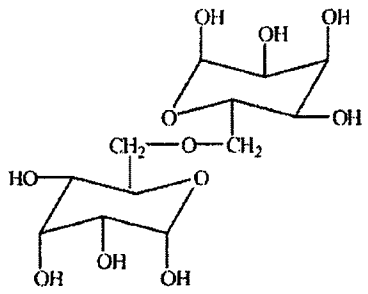


7 8

Brickellia veronicaefolia



Arocomia mexicana



9 10

Fig. 1. (*Continued*).

while throughout the progression of diabetes high levels of circulating radical scavengers have been recorded (Letitia et al., 2002).

Many of the complications of diabetes, including retinopathy and atherosclerotic vascular disease, the leading cause of mortality in diabetics, have been linked to oxidative stress (Baynes, 1991). In diabetic patients, isoorientin decreases the circulating of radical scavengers, and reduces symptoms of associated complications. However, the hypoglycaemic effect of this compound has not yet been tested.

3.1.7. Toxicity

In the previously mentioned work by Pérez-Gutiérrez et al. (2001) the acute toxicity was tested in Swiss mice. The

authors conclude: "the median lethal dose (LD_{50}) of aqueous extract from *Cecropia obtusifolia* after i.p. administration is 1450 mg/kg animal (11.21 g of plant/kg of weight)". This is the equivalent to 673 g for a 60 kg person, far higher than the commonly used dose of 15 g per person and day. The authors conclude that the aqueous extract of the plant has low toxicity.

3.1.8. Cecropia obtusifolia—conclusion

Some evidence mostly from *in vivo* animal studies is available which validate the use of *Cecropia obtusifolia* in diabetes. More studies are needed on type 2 diabetic animals and in patients to elucidate the complete hypoglycaemic mechanism of *Cecropia* extract. The extract has two main bioactive compounds, chlorogenic acid may well be responsible in part for the observed effect—the strongly reduced glucose production by the liver in a fasting state. However, since Andrade-Cetto and Wiedenfeld (2001) and Román-Ramos et al. (1991) reported an early hypoglycaemic effect, this action cannot be due to chlorogenic acid. Furthermore studies focusing on chronic application over longer time periods (at least one or two months) may also help to elucidate the mechanism of action. In such a study, data on the insulin production should be recorded, too. An extract from this species has a great potential to be further developed into a phytomedicine to treat type 2 diabetes in humans.

3.2. Equisetum myriochaetum Schlecht & Cham (Equisetaceae)

The plant is sold in several markets in México to treat kidney diseases (mal de orin) and diabetes.

3.2.1. Botanical description

Terrestrial plant with aerial stems 2–5 m (to 8 m) high, branched with regular verticilies 2–23 mm in diameter with 16–48 channels, terminal strobile in the branches and in the main stem 10 mm long and 4 mm in diameter (Palacios-Rios, 1999).

3.2.2. Distribution

It is known from the following Mexican states: Nayarit, Michoacán, Guerrero, Nuevo Leon, San Luis Potosí, Tamaulipas, Hidalgo, Puebla, México, Veracruz, Oaxaca and Chiapas. Again, it is a weedy species and there seems to be ample opportunity for collecting material form this species in a sustainable way.

3.2.3. Ethnobotany

Species of equisetum, mainly *Equisetum hymale*, *Equisetum laevigatum* and *Equisetum myriochaetum*, are traditionally used against kidney diseases. They are sold indistinctly on the markets. Traditionally, a decoction of the aerial part of the plant is prepared and consumed as "Agua de uso" (Argueta, 1994) The use as treatment of type 2 diabetes was described by Andrade-Cetto et al. (2000), the form of preparation is the same as for *Cecropia obtusifolia*.

3.2.4. Main constituents

From the butanolic and the water extracts with hypoglycaemic activity the following constituents were isolated: kaempferol-3-*O*-sophoroside, kaempferol-3,7-di-*O*-β-glucoside, caffeoyl-methylate-4-β-glucopuranoside and kaempferol-3-*O*-sophoroside-4ï-*O*-β-glucoside (Fig. 1, compound 3, Wiedenfeld et al., 2000). Pinocembrin, chrysin, β-sitosterol, β-D-glycosyl-sitosterol, β-D-glucose and fatty acids were also mentioned as constituents of *Equisetum myriochaetum* (Camacho et al., 1992).

3.2.5. Pharmacology

The hypoglycaemic effect was demonstrated in Streptozotocin diabetic rats (Andrade-Cetto et al., 2000, and in diabetic type 2 patients (Revilla-Monsalve et al., 2002). Although the plant is reported mainly for kidney diseases it showed a remarkable hypoglycaemic effect in both tested models. There already exist reports about hypoglycaemic activities of various kaempferol derivatives containing plant extracts: Kaempferol 3-*O*-galactoside and Kaempferol 3-rhamnoglucoside from *Bauhinia variegata* (Andrade-Cetto, 1999), Kaempferol 3-*O*-rhamnoside from *Zizhyphus rugosa* (Khosa et al., 1983), Kaempferol 3-*O*-beta-glucopyranoside from *Morus insignis* (Basnet et al., 1993), and Kaempferol-3-*O*-(2gal-rhamnosilobonoside) from *Sterculia rupestris* (Desoky and Youssef, 1997).

A lower risk of type 2 diabetes has been associated with flavonoid intake specially quercetin and myrcetin (Knekt et al., 2002). The authors suggest an inverse association between flavonoid intake and subsequent occurrence of ischemic heart disease, cerebrovascular disease, lung and prostate cancer, type 2 diabetes, and asthma. The potential beneficial effect was associated with quercetin (the strongest antioxidant) but also with kaempferol.

The pharmacological testing in Streptozotocin diabetic rats showed a significant activity from 60 to 180 min, for the water and the butanolic extract. The most potent effect was shown by kaempferol-3-*O*-sophoroside-4′-*O*-β-glucoside. The water extract was also tested in type 2 diabetic patients. The results obtained in this study show a significant effect on the reduction of the glucose levels in these patients after the oral administration of an *Equisetum myriochaetum* water extract. The hypoglycaemic effect started 90 min after the administration of the decoction and was maintained for another 90 min. Insulin levels did not significantly change during the study, implying that the mechanism of action is not glibenclamide-like (not due to stimulation of insulin secretion).

3.2.6. Toxicity

In experiments performed with 200 male of *Drosophila melanoganster* (flr3/TM3,BdS), the traditionally used aqueous extract did not shown any toxicity, in up to 3700 ppm no LD_{50} was observed (Bárcenas-Rodríguez, 2004). The relevance of these data is, of course, limited.

3.2.7. Equisetum species—conclusion

The antioxidant effect of flavonoids cannot explain the acute effect of the plant. For developing a more widely used phytomedicine for use in type 2 diabetes, more studies are urgently required.

3.3. Acosmium panamense (Benth.) Yacolev (Fabaceae)

This species is widely used especially in the southern lowland of México for treating fever, malaria and in recent decades, diabetes (Heinrich, 1989).

3.3.1. Botanical description

Acosmium panamense (Benth.) Yacolev, (syn, *Sweetia panamensis* Benth, with traditional names "Guayacán" and "Bálsamo amarillo") is a tree up to 40 m height, growing in the tropical rain forest as a co-dominant species with *Terminalia amazonia* and *Vochysia guatemalensis* (Pennington and Sarukhán, 1998). The main characteristic of the tree is a tall, straight trunk pyramidal treetop with ascendant branches. The external cortex is plain and dark grey, the inner cortex is yellow and bitter. Leaves obtuse and pubescent surrounded by stipules with a spiral disposition. Fruit green to dark green legumes, 5–10 cm long (Pennington and Sarukhán, 1998).

3.3.2. Distribution

It grows along the Gulf coast from Veracruz to Yucatan and along the Pacific from Oaxaca to Chiapas. It is a co-dominant species from the tropical rain forest. It is often managed by local people (Heinrich, unpublished data), seems to be quite abundant, but no information on the potential of sustainable harvesting, especially if the bark is to be used, is available.

3.3.3. Ethnobotany

In Oaxaca the plant is used traditionally for the treatment of stomach pain, respiratory problems, diarrhoea, malaria and "marsh fever". The plant medicine is prepared as an infusion of the bark and it is taken orally 1–2 times per day. In addition, *Acosmium panamense* is utilized to treat diabetes in the village of Soteapan, Veracruz (Leonti et al., 2001; Leonti, 2002), and in Oaxaca (Andrade-Cetto and Wiedenfeld, 2004; Heinrich, 1989; Heinrich et al., 1992).

3.3.4. Main constituents

Phytochemical studies of the plant yielded several quinolizidine alkaloids like acosmine and acosminine, hydroxysparteine as well as lupinane alkaloids (Balandrin and Kinghorn, 1982; Argueta, 1994; Veitch et al., 1997; Nuzillard et al., 1999). From the water extract of the traditionally used bark (Wiedenfeld and Andrade-Cetto, 2003) caffeic acid and three pyrones were isolated: desmethylyangonine its $O^{4'}$-mono as well as the di(1–6)glucoside (Fig. 1, compounds **4–6**).

3.3.5. Pharmacology

The water and butanol extract as well as a mixture of the isolated substances (4 and 5) were tested in Streptozotocin diabetic rats. For all tested extracts the hypoglycaemic effect was statistically significant with respect to the control at 120 and 180 min. The main constituents of the traditionally used water extracts are the isolated pyrones, similar pyrones are found in *Piper methysticum* Forst. f. (Kava Kava) used until recently as (often licensed) phytomedicines for the treatment of anxiety disorders: 11-methoxy-5,6-dihydroyangonin, 11-methoxytetrahydroyangonin, tetrahydroyangonin, desmethoxyyangonin and yangonin, with the last two being the most abundant, (Ranjith et al., 2002). These compounds had previously not been evaluated for hypoglycaemic activity.

3.3.6. Acosmium panamense—conclusion

Limited *in vivo* evidence exists for the traditionally used water extract. The isolated pyrones have hypoglycaemic activity, but more studies are needed to clarify the mode of action.

3.4. Cucurbita ficifolia Bouché (Cucurbitaceae)

3.4.1. Botanical description

At the end of the 19th and the beginning of the 20th century, some authors were suggesting an Asiatic origin for *Cucurbita ficifolia*. Since the middle of the last century, the consensus has been that it is of American origin. However, its centre of origin and domestication are still unknown. Some authors have suggested Central America or southern México as places of origin, while others suggest South America, and more specifically the Andes (Purdue University, 2004).

Cucurbita ficifolia is a creeping or climbing plant, monoecious, annual, up to 10 m long. It is villose to softly pubescent with some short sharp spines dispersed over the vegetative parts. It has five vigorous, slightly angular stems and ovate-cordate to suborbicular-cordate leaves with 5–25 cm long petioles. The flowers are pentamerous, solitary, and axillary. The fruit is globose to ovoid-elliptical. The flesh is sweet and the seeds are ovate-elliptical, flattened, and of a dark brown to black or creamy white colour (Purdue University, 2004).

3.4.2. Ethnobotany

The popular name for the plant is "Chilacayote". The fruit, is used externally to treat a worm that runs under the skin (like *larva migrans*) in Hidalgo (Argueta, 1994). In México, the plants is consumed widely and several dishes and candies are prepared with the seeds or fruit. Aguilar et al. (1994) summarise the use of the fruit as a treatment of diabetes: the healers recommend the ingestion of the fruit macerated in water.

3.4.3. Main constituents

Lectins were isolated from stems and roots of 6-day old seedlings by precipitation with ethanol, affinity chromatography on Con A-Sepharose, gel filtration on Bio-gel P100 and separated by electrophoresis on polyacrylamide gel. Three purified lectins (RLA(1), RLA(2), RLA(3)) were obtained

from roots and four from stems (SLA(1), SLA(2), SLA(3), SLA(4)) (Lorenc-Kubis et al., 2001). Acosta-Patino (2001) reports 90% of edible portion, 94% moisture, 0.3% fibre content, 1.2% protein, 17 mg calcium, 0.6 mg iron, 7 mg ascorbic acid, 0.03 mg thiamine, 100 g of *Cucurbita ficifolia* produces 3.34 Kjoule (14 Kcal). However, there is no report about the main constituents of the fruit extract.

3.4.4. Pharmacology

The pharmacological activity of the plant was tested in hyperglycaemic rabbits (Román-Ramos et al., 1991). The rabbits were submitted to glucose tolerance test and a preparation of the plant or tolbutamide was administrated, the animals receive 2 g/kg of glucose subcutaneously at the starting point and 60 min later. Water was used as control. The authors report a statistically significant hypoglycaemic effect of the plant from 60 min until 300 min. The amount of extract and the way of preparation are not reported in the paper.

Several experiments were performed by Alarcón-Aguilar et al. (2002) in alloxan induced mice and rats. Mature fruits of *Cucurbita ficifolia* were cut in halves. The juice was obtained with an electric extractor and freeze-dried. The acute effect was tested in *healthy* mice using two routes of administration, oral (po) and intraperitoneally (i.p.) at 500 mg/kg. In case of the po administration, the authors report an hypoglycaemic effect at 240 min with $p<0.05$, while with the (i.p.) they observe statistically significant activity at 120 and 240 min. The acute effect was tested with the (i.p.) administration of the extract at 25, 250, 500, 594. 750, 1000, 1250 mg/kg, the hypoglycaemic effect was observed at 120 min with $p<0.05$ for doses down to 750 mg and $p<0.001$ for 1000 and 1250 mg at 240 min with $p<0.001$ for all the doses, all compared with the control group. In alloxan diabetic mice, the acute effect was also tested at 500 mg/kg (i.p.), the authors report a hypoglycaemic effect with $p<0.001$ at 120 and 240 min. Tolbutamide was used as control drug. The daily administration of 1000 mg/kg to alloxan diabetic rats, resulted in a gradual reduction of the blood sugar levels, at days 7 and 14, when the measures were taken.

In 2001, Acosta-Patiño tested the effect of the fruit juice in patients with moderate hyperglycaemia at 4 ml/kg (100 g of fruit = 75 ml of juice), Blood glucose levels were analyzed hourly during 5 h using a commercial enzymatic kit. In another session, at least separated by 8 days, the same group of patients received, the same amount of potable water as control. The authors report the hypoglycaemic effect at 180 min with $p<0.05$, at 240 min with $p<0.01$, and at 300 min with $p<0.001$.

3.4.5. Toxicity

Some toxicity has been detected in the majority of the hypoglycaemic Cucurbitaceae species (Marles and Farnsworth, 1995) often due to cucurbitacines. The results from Alarcón-Aguilar (2002) showed that freeze-dried juice of *Cucurbita ficifolia* fruits had toxicity when administered intraperitoneally to mice and when it was orally administered daily for 14 days to alloxan-diabetic rats, the LD50 was 650 mg/kg with limits of 518.2 and 753. 8 mg/kg, while the administration of 1250 mg/kg cause the death of 100% of the animals.

3.4.6. Cucurbita ficifolia—conclusion

The fruit showed a hypoglycaemic activity in all the reported studies, the lack of phytochemical information on the juice (extract), prevents an assessment of the observed effect on a phytochemical level. In the clinical study, the authors conclude: "Due to the negligible content of fiber in *Cucurbita ficifolia* and the design of the study, the observed effects on glucose levels are not a consequence of glucose absorption changes in the intestine" (Acosta-Patino, 2001). In all cases, the doses used were high and the therapeutic relevance of this effects has to be questioned.

Extrapolating the toxicity levels reported in Alarcón-Aguilar (2002) to reach the lethal dose of 1250 mg/kg obtained from the freeze-dried juice, a person of 60 kg would need 75,000 mg (75 K) of fruit to have the lethal dose, and this is much higher than the traditionally recommended dose of 32 g. More studies are needed in order to identify the constituents of the fruits, and then test these substances. Quantitative phytochemical studies on the levels of lectins and other potentially relevant constituents during the development of the various organs of *Cucurbita ficifolia* and their link to potential toxic effects should also be conducted.

3.5. Agarista mexicana (Hemsl.) Judd (Ericaeae)

3.5.1. Botanical description

Shrub or tree to 8 to 11 m tall, with thick, corky, deeply furrowed bark; twigs very sparsely to densely pubescent, with nonchambered to clearly chambered pith; buds to ca. 1.5 mm long, leaves revolute. Inflorescences (fascicle-like) axillary racemes, flowers with triangular calyx lobes, with acuminate apices, capsules subglobose to short-ovoid (NYBG, 2004).

3.5.2. Distribution

Mountainous areas of México and Central America, from Veracruz and Jalisco south to Quintana Roo.

3.5.3. Ethnobotany

The water extract of the leaves of this plant known as "Palo Santo" is used orally to treat diabetes (Pérez-Guerrero et al., 2001).

3.5.4. Main constituents

From the chloroform extract of the dried steam of the plant, 12-ursene and the triterpene-23,24-dimethyl 1-24-ethyl-sigmast-25-ene were isolated (Fig. 1, compounds 7 and 8).

3.5.5. Pharmacology

Blood glucose levels of normal and alloxan-treated diabetic mice and rats were determined after oral administra-

tion of the chloroform extracts of *Agarista mexicana* at 100 and 150 mg/kg. The oral administration of the extracts produced a significant hypoglycaemic effect in normal as well as in diabetic mice and rats (Pérez-Gutiérrez et al., 1996). The effect of the isolated compounds was tested in alloxan induced diabetic and in normoglycaemic mice at 50 mg/kg (i.p.). Compound **6** showed an statistically significant activity at 90 min, 180 min, 270 min and 1440 min (24 h), in alloxan diabetic mice, while the effect in normoglycaemic mice was observed at 90 and 270 min. Compound **7** shows statistically significant activity at 180 and 270 min in alloxan diabetic mice, and at 90 and 18 min in normoglycaemic mice, all this against the control groups. Tolbutamide was used as positive control (Pérez-Gutiérrez and Vargas, 2001).

3.5.6. Agarista mexicana—conclusion

The hypoglycaemic effect of the extract has been demonstrated and two terpenes were isolated as bioactive compounds, similar ursine triterpenes were isolated from the water-chloroform extract of the roots of *Tripterygium wildfordii* a traditional Chinese plant used against rheumatoid arthritis and other inflammatory and autoimmune disorders. However, the reported effect is of the total multi-glycoside extract, in which the ursenes are present together with other compounds (Duan et al., 2001). The therapeutic effect of the plant is attributed to the extract and not to a single compound. The mechanism of action of the isolated 12-ursene and the 23,24-dimethyl 1-24-ethyl-sigmast-25-ene is currently not known.

A comparison between the water extract (traditionally used) and the chloroform extract tested (e.g. regarding the presence of the bioactive compounds) would be of considerable interest. It is necessary to know the amount of bioactive compounds in the traditional tea, also toxicological testing is required in order to ensure the safety of the plant.

3.6. Brickellia veronicaefolia (Kunth) A. Gray (Asteraceae)

3.6.1. Botanical description

Bush 40 cm to 1 m tall, branched at the base with grey-red stems, white or pink flowers, present at the union of the stem and leaves.

3.6.2. Ethnobotany

The plant is know as "oregano de monte" the main use is against gall problems, especially bile, other uses are against stomach pain. For the later, the branch is boiled in water and a bitter infusion results (Argueta, 1994). The use against diabetes is reported in Pérez-Gutiérrez et al. (1998).

3.6.3. Main constituents

The following constituents have been isolated from the leaves: flavones – artementin, brickellin, casticin and trimetoxiquercetagenin, flavonols, eupatin, eupatolin, patuletin and vernicaefolin, and labdane diterpens (Argueta, 1994). From the chloroform extract Pérez et al. (2000) isolate the bioactive flavone 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavone (Fig. 1, compound **9**).

3.6.4. Pharmacology

A chloroform extract of the leaves was tested in alloxan diabetic mice and normoglycaemic mice (i.p.) at 100, 200 and 300 mg/kg. The extract showed significant activity with at least $p < 0.01$ in both models for all the tested doses (at 90, 180, 270 and 1440 min = 24 h; Pérez-Gutiérrez et al., 1998). In the same models, the isolated flavone was tested (i.p. 10, 25 and 50 mg/kg). In the alloxan diabetic mice, 10 mg/kg shows statistically significant activity at 90, 270 and 1440 min with $p < 0.05$, 25 mg/kg shows statistically significant activity at 90 and 180 ($p < 0.01$) min, and at 270 and 1440 min ($p < 0.05$). 50 mg/kg shows activity only at 270 and 1440 min. In the normo-glycaemic mice, 10 mg/kg showed activity only at 270 min, 25 mg/kg was active at 90 and 180 min ($p < 0.01$) and at 1440 min ($p < 0.05$). 50 mg/kg showed activity at 90 and 180 min ($p < 0.01$) and at 1440 min ($p < 0.05$). In alloxan diabetic mice, the maximum effect observed was at 270 min, compared to the control groups. In all tests, tolbutamide was used as control.

3.6.5. Brickellia veronicaefolia—conclusion

The hypoglycaemic effect was confirmed and a bioactive compound has been isolated. The possible hypoglycaemic effect of flavonoids has been discussed above, there are no other reports of the isolated flavone. Toxicological test, as well as a comparative phytochemical investigation of the traditionally used and the tested chloroform extract would be highly desirable. The active doses reported by the authors are too high for use in traditional medicine or as a phytomedicine. In case of the chloroform extract, a person of normal weight (60 kg) would need 85 g of plant to get the desired effect.

3.7. Parmentiera aculeata (Kunth) Seem. (Bignoniaceae)

The fruit of the tree is reported to be hypoglycaemic, however the reports (Pérez-Gutiérrez et al., 1998, 2000a) discuss the species under a synonym: *Parmentiera edulis* DC.

3.7.1. Botanical description

Tree up to 15 m, branched since the base and channelled trunk, external cortex dark yellow with fissures and scaly ribs. The fruit is a berry up to 15 cm long by 6.5 cm wide, with several longitudinal furrows and a green-yellow colour.

The species is managed by humans to produce shade and is a widely distributed species known along the Gulf and Pacific coast from Tamaulipas to Yucatán and from Sinaloa to Chiapas (Pennington and Sarukhán, 1998).

3.7.2. Ethnobotany

The fruit and the cortex bark of the tree are boiled in water to treat kidney diseases, and for the treatment of diabetes

(Argueta, 1994). The plant is used in Guatemala to treat gonorrhoea (Caceres et al., 1995).

3.7.3. Main constituents

The guaianolide of lactucin-8-*O*-methylacrylate was isolated from the chloroform extract of the fruit. The hypoglycaemic activity was reportedly associated with this compound (Pérez et al., 2000). From the bark beta-sitosterol and tannins are reported (Argueta, 1994).

3.7.4. Pharmacology

The chloroform extract of the fruit was tested intraperitoneally in alloxan diabetic mice CD1 (strain) at 100, 200 and 300 mg/kg. For the dose of 100 mg/kg they report significant effects compared to the control at all observed times [90,270, 180 and 1440 (24 h) min]. For 200 mg/kg they also report effect at the same times, while the dose of 300 mg/kg shown also the same effects. However the different doses did not shown different effect.

Similar effects were observed in normoglucemic mice (Pérez-Gutiérrez et al., 1998c). Lactucin-8-*O*-methylacrylate isolated from the active fraction was tested (i.p.) on alloxan diabetic mice CD1. Again the data is reported as glucose reduction percent. At a dose of 10 mg/kg the authors report an significant hypoglycaemic activity at 90, 180 and 1440 min. Statistical significance was missed at 270 min. At a dose of 25 mg/kg significant activity is observed at 270 and 1440 min only, while at a dose of 50 mg/kg the activity is observed at 90, 180 and 270 min against the control group. Tolbutamide was used as reference (Pérez et al., 2000).

3.7.5. Parmentiera aculeate—conclusion

The hypoglycaemic effect of the chloroform extract and the isolated compound has demonstrated by the authors. However, the data (glucose values) of what happened between 270 and 1440 min is not reportedfor all the experiments. According to the data reported, the dose of 300 mg/kg of the fruit used in the first experiment, will be equivalent that a 60 kg person would have to eat 18 g of dried fruit for get the desired effect, which is to much for a single dose.

More studies are needed to know how the extract is working, in witch amount the isolated compound is present in the fruit, and as in previous examples there is no information on the bioavailability of the drug. Toxicity studies are also needed in order to develop a Phytomedicine.

3.8. Other species

As indicated in Table 1 many other species are commonly used in México. Some have received some attention in pharmacological and phytochemical studies.

Arocomia mexicana (Arecaecea), From the methanol extract of the root Pérez et al., 1997 isolated Coyolosa (Fig. 1, compound **9**) a tetrahydropyrane. The compound was tested on alloxan induced hyperglycemic mice and rats, at doses of 5.0–20 mg/kg i.p. the coyolosa exhibited significant blood sugar lowering at 1.5, 3.0, 4.5 and 24 h against the untreated control.

*Verbesina persicifoli*a DC. (Asteraceae; chloroform extracts at 100 mg/kg and 150 mg/kg) was tested in normal and alloxan diabetic mice. The authors conclude that those doses produced a significant hypoglycaemic effect in normal as well as in diabetic mice and rats (Pérez-Gutiérrez et al., 1996).

A hexane extract from *Cirsium pazcuarense* (Kunth) Spreng, (reported in the original paper as *Cirsium pascuarense*) at 100, 150 and 200 mg/kg i.p. showed a significant hypoglycaemic effect in normal as well as in diabetic mice. In addition, the extract altered glucose tolerance in alloxan induced diabetic rats. Chloroform and methanol extracts did not produce any significant change in blood glucose levels (Pérez et al., 2001). This is an example highlighting the need for proper taxonomic validation of a botanical identification.

The acute effects of the freeze-dried decoction of the roots of *Ibervillea sonorae* (S. Watson) Greene (Cucurbitaceae) on blood glucose levels were investigated in fasting mice. The authors report that: "the plant orally administrated to healthy mice did not cause a significant decrease of the blood glucose level. However, *Ibervillea sonorae* reduced the blood glucose of normal mice in a dose-dependent manner after intraperitoneal injection ($P < 0.05$). Also, this extract significantly lowered the glycaemia of mild alloxan-diabetic mice and rats, but did not in severe alloxan-diabetic rats, so it seems that this antidiabetic plant needs the presence of insulin to show its hypoglycaemic activity. Chemical, pharmacological, and toxicological investigations of *Ibervillea sonorae* must continue to establish its use as an alternative in the control of diabetes mellitus" (Alarcón-Aguilar et al., 2002).

Of note, at least three of the species discussed above are edible fruits. In Table 1, a large number of other food plants (most notably vegetables) are included: *Allium cepa* L. (Cebolla), *Ananas comosus* (L.) *Merr.* Piña), *Annona cherimola Mill.*, (Chirimoya), *Arachis hypogaea* L. (Cacahuate), *Asclepias linaria Cav.* (Romerillo), *Byrsonima crassifolia* (L.) *Kunth* (Nanche), *Carica papaya* L. (Papaya), *Casimiroa edulis* La Llave & Lex. (Zapote blanco), *Citrus aurantifolia* (*Christm.*) *Swingle* (Naranjo), *Citrus limetta* Risso (Lima), *Coriandrum sativum* L. (Cilantro), *Costus ruber* C. Wright ex Griseb. (Caña agria), *Crataegus pubescens* (C. Presl) C. Presl (Tecojote), *Cucurbita ficifolia* (L.) Bouché (Chilacayote), *Cucurbita mexicana Damm.* (Calabaza), *Cynara scolymus* L. (Alcachofa), *Daucus carota* L. (Zanahoria), *Eriobotrya japonica* (Thunb.) Lindll. (Níspero), *Leucaena leucocephala* (Lam.) *de Wit* (Guaje), *Nopalea cochenillifera* (L.) *Salm-Dyck* (*Nopal*), *Nopalea indica* L. (Nopal), *Persea americana Mill.* (Aguacate), *Petroselinum crispum* (Mill.), *Nyman ex A.W. Hill* (Perejil), *Phaseolus vulgaris* L. (Frijol), *Physalis philadelphica* Lamm (Tomate), *Piper auritum Kunth* (Hierba santa), *Portulaca oleraceae* L. (Verdolaga), *Psidium guajava* L. (Guayaba), *Sechium edule* (Jacq.) Sw. (Chayote), *Solanum verbascifolium Banks ex Dunal* (Berenjena), *Tamarindus indica* L. (Tamarindo). Phytochemically

these are very diverse taxa, but the importance of such fruit certainly highlights the health beneficial effects of a diet rich in plant fibre. While currently specific pharmacological effects of this diverse group of species cannot be ascertained, it is possible that modification of the passage time or changes in the GI flora have an indirect influence. This opens a fascinating are of research at the interface of food and medicines (cf. Heinrich, 1998).

4. General conclusion

Clearly, a large number of species are used in today's México to treat diabetes or its symptoms. An interesting and unresolved issue relates to the way such uses were developed over the last decades. It seems that many of the species were originally used for a variety of kidney disorders and most notably for their diuretic effect. From an ethnopharmacological perspective, it would be extremely interesting to analyse this process further.

For further testing proper animal model have to be used (Versphol, 2002). Today, the models used resemble type 1 diabetes or are no models for diabetic testing like glucose overload. The only model with supporting data for type 2 diabetes is the Streptozotocin diabetic rat (Islas-Andrade et al., 2000). But according to Versphol (2002), the only way to get type 2 diabetic animals by chemical induction is by the proper use of Streptozotocin in neonatal rats (n-STZ), or use genetically models like fa/fa Zucker diabetic fatty rat. None of these models has been used until now to test Mexican plants, and according to the group of Pérez-Gutierrez et al. a method developed in 1964 is the most commonly used by them, so also there needs to be an update of the pharmacological tools we use.

We propose three levels of intervention led by the goal to reduce the public health impact of this syndrome involve government actions at all levels.

Nutritional education of the general population is a first step which could reduce the epidemic proportion of the disease. One core problem is the high consumption of sweet drinks commonly called "refrescos" all over México. When conducting field work in any region of México one cannot fail to note large quantities of discarded plastic bottles in each back yard. Also the consumption of such "referescos" is visible everywhere and at anytime. Therefore, strict regulations about the content of sugar in those drinks would be highly desirable (e.g. via a special sales tax), and of course it would be ideal to largely avoid such beverages. The government and health professionals should also promote exercise among people living in the cities, to avoid sedentary way of life.

Additionally, there must be some efforts to monitor and control the plants sold on markets and widely collected by the people for autoconsumption. Educational programmes together with pharmcodynamic studies should have first priority. The latter research should include projects on the species' mechanism of action, on the optimal doses and treatment schedule, and on the best mode of preparation. Even though pharmacoeconomic studies on the costs of such treatments are lacking, it is likely that, for example, cost of treatment with *Cecropia* leaves bought on the Mercado de Sonora in México, DF may easily reach MEX$ 250 (US$ 20) per month of treatment. This highlights another important point – the economic impact of using such herbal remedies has not been studied at all, but it is likely to be an important cost factor for many poorer families. The production of medicinal teas or simple preparations with ascertained quality that could be sold on markets should be promoted as part of this intervention. Such an approach would assure a health beneficial effect of the final product. We see efforts like this, for example on the market in Mérida, Yucatán (Andrade-Cetto., pers. obs.), where a healer is selling an ethanolic preparation of *Malmea depressa* (drops). As a next step simple quality control measures could be established. These initiatives must again be accompanied by the appropriate training and education programmes directed at diabetics, physicians and social workers to ensure that the people drink those preparations in a medically and pharmaceutically appropriate way. In order to achieve this we still have a long way to go and México with its rich tradition in medicinal plants use still lacks appropriate training for physicians and pharmacists in phytotherapy and phytopharmacy.

The third level on our opinion the most important one. It focuses on the development of a phytomedicine with hypoglycaemic effects at early stages of the disease or even prior to the start of the disease (during the period of increased insulin resistance). In this context, the isolation of the main compounds from the active extract is a crucial step in all R&D activities for developing a novel phytomedicine. The use of a phytomedicine is suggested because it would be subject to quality control, and could be prescribed by physicians. Herbal drugs are mainly whole, fragmented or cut, plants parts of plants, algae, fungi, lichen in an unprocessed state, usually in a dried form, but sometimes fresh. They are precisely defined by their botanical (scientific) binomial (Heinrich et al., 2004). The herbal drug preparation (phytomedicine) is obtained subjecting herbal drugs to treatments such as extraction, distillation, fractionation, purification, concentration and fermentation. These include cut or powered herbal drugs, tinctures, extracts, essential oils, fatty oils, expressed juices and processed exudates (Gaedcke and Steinhoff, 2003). Clearly, considerable research will be required for developing such products which could be of enormous benefit to the Mexican population suffering from a drastic increase in this chronic and debilitating disease. In comparing México with the examples of Germany or France, where the phytomedicine market moves billions of dollars each year, it becomes apparent that Mexican businessmen have an opportunity to develop such novel products. Alternatively, the Mexican Social Security System, which has for my years conducted research on popularly used medicinal plants, could take a lead and develop phytomedicines which would be available at relatively low cost.

A difficult and unresolved issue relates to the traditional intellectual property on these species. Clearly, they have been used widely in México, knowledge about these these species, their bioactive compounds and pharmacological effects is in the public domain, their use in the systematic treatment of diabetes is relatively recent and it will be nearly impossible to identify one group of people which can claim traditional ownership. However, since some of these phytomedicines may also be commercialised outside of México, the Mexican government or other appropriate institutions will have develop ways to guarantee the sustainable use of this species and that any economic benefit from these phytomedicines will also be shared with the Mexican people.

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